Measuring engineered nanomaterial toxicity in biological systems

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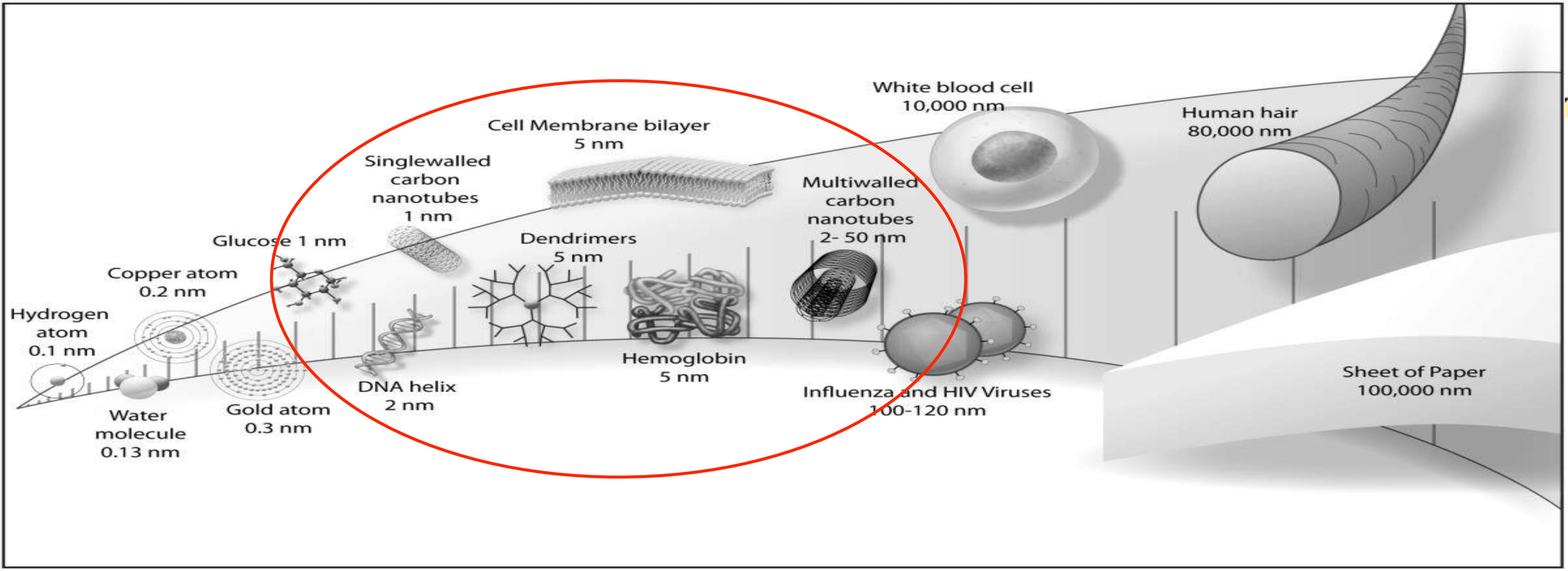
Burgos 2nd October 2017

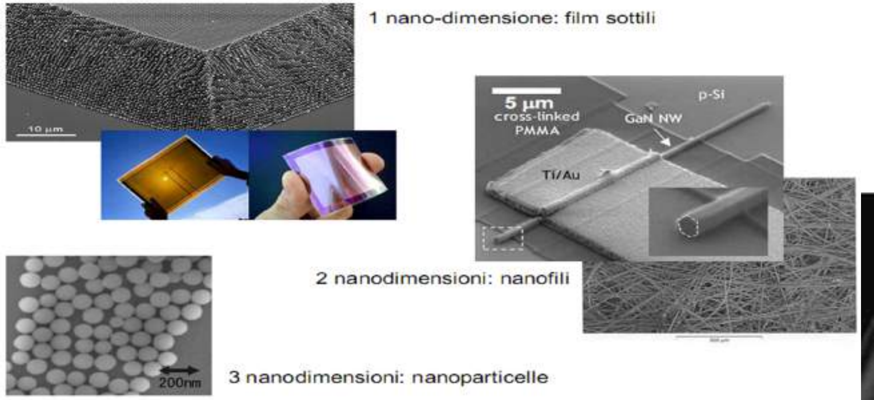


Fonte:
Yokel and MacPhail, 2011

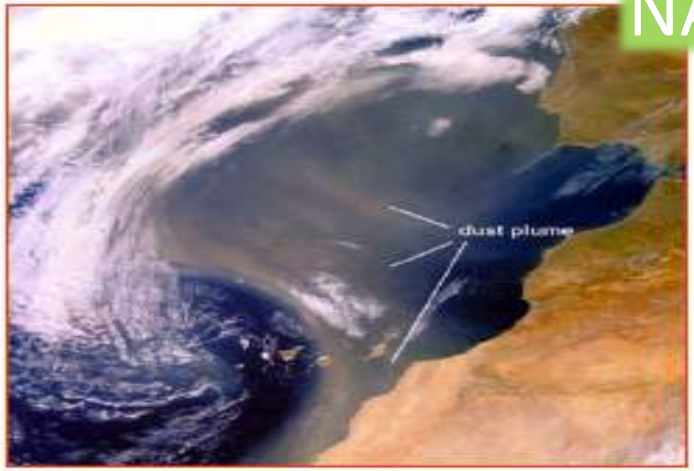
NANOGENTOOLS

Nanotechnology: science of manipulating matter at the molecular scale and holds the promise of providing significant improvements in technologies, including industrial manufacturing, human health, medicine, personal care, and even environmental protection

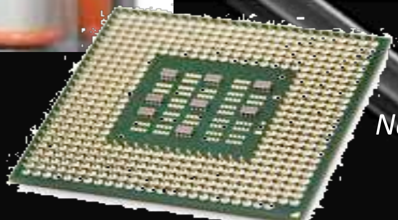
Nanomaterials: at least one dimension between 1 and 100 nm



NATURAL NANOPARTICLES



ENGINEERS NANOPARTICLES



NANOGENTOOLS

Iron Oxide as an example of naturally occurring NM

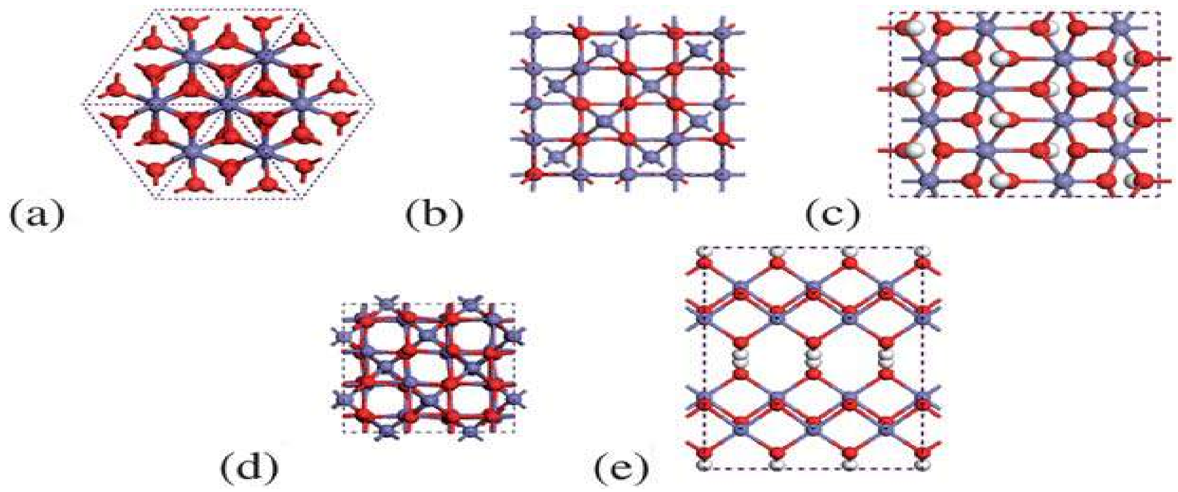


Fig. 1 Schematic representation of the most abundant iron oxide and oxy-hydroxides: (a) hematite, (b) magnetite, (c) goethite, (d) maghemite, and (e) lepidocrocite. Ferrihydrite is not shown as the structure is still under debate in the literature. All structures are viewed from the ⟨001⟩ or ⟨0001⟩ directions.

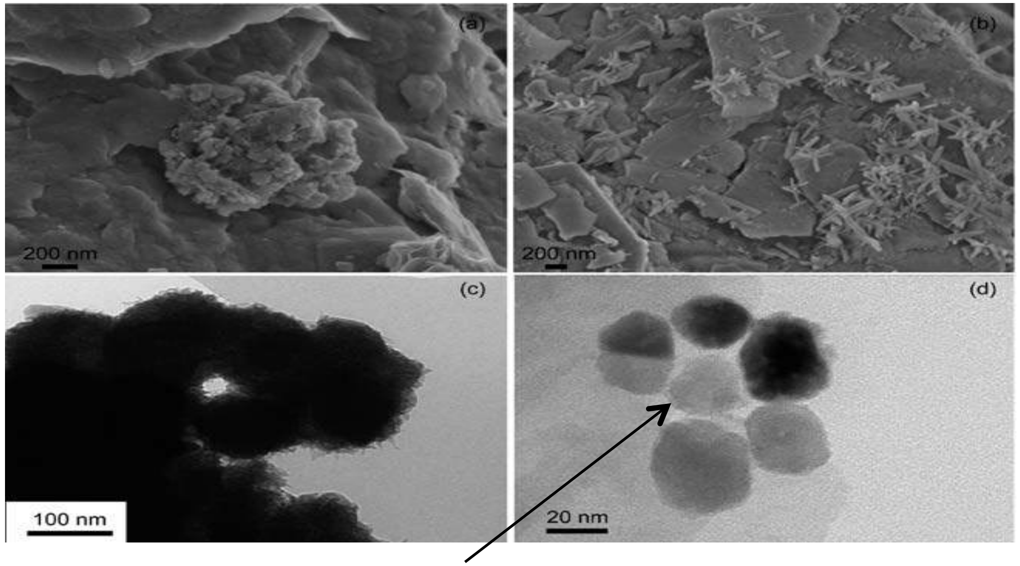
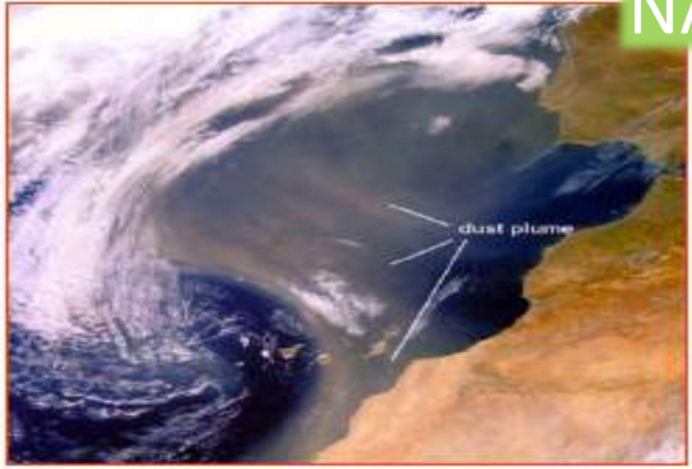


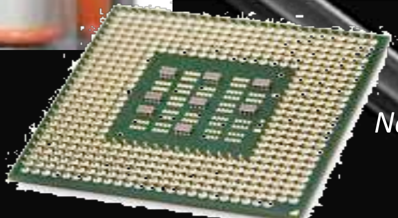
Fig. 2 Iron oxide nanoparticles in iceberg-hosted sediments: (a) irregular shaped aggregate of ferrihydrite, (b) acicular twinned goethite, (c) schwertmannite pin-cushion spheroidal aggregates, and (d) hematite nanoparticles of irregular rounded shapes. (a–c) are from ref. 12, Copyright (2011), (d) is from ref. 13, Copyright (2011); both with permissions from Elsevier.

J. Mater. Chem. A, 2013, **1**, 27–42 |

NATURAL NANOPARTICLES



ENGINEERS NANOPARTICLES



Nanogen

ENM facts

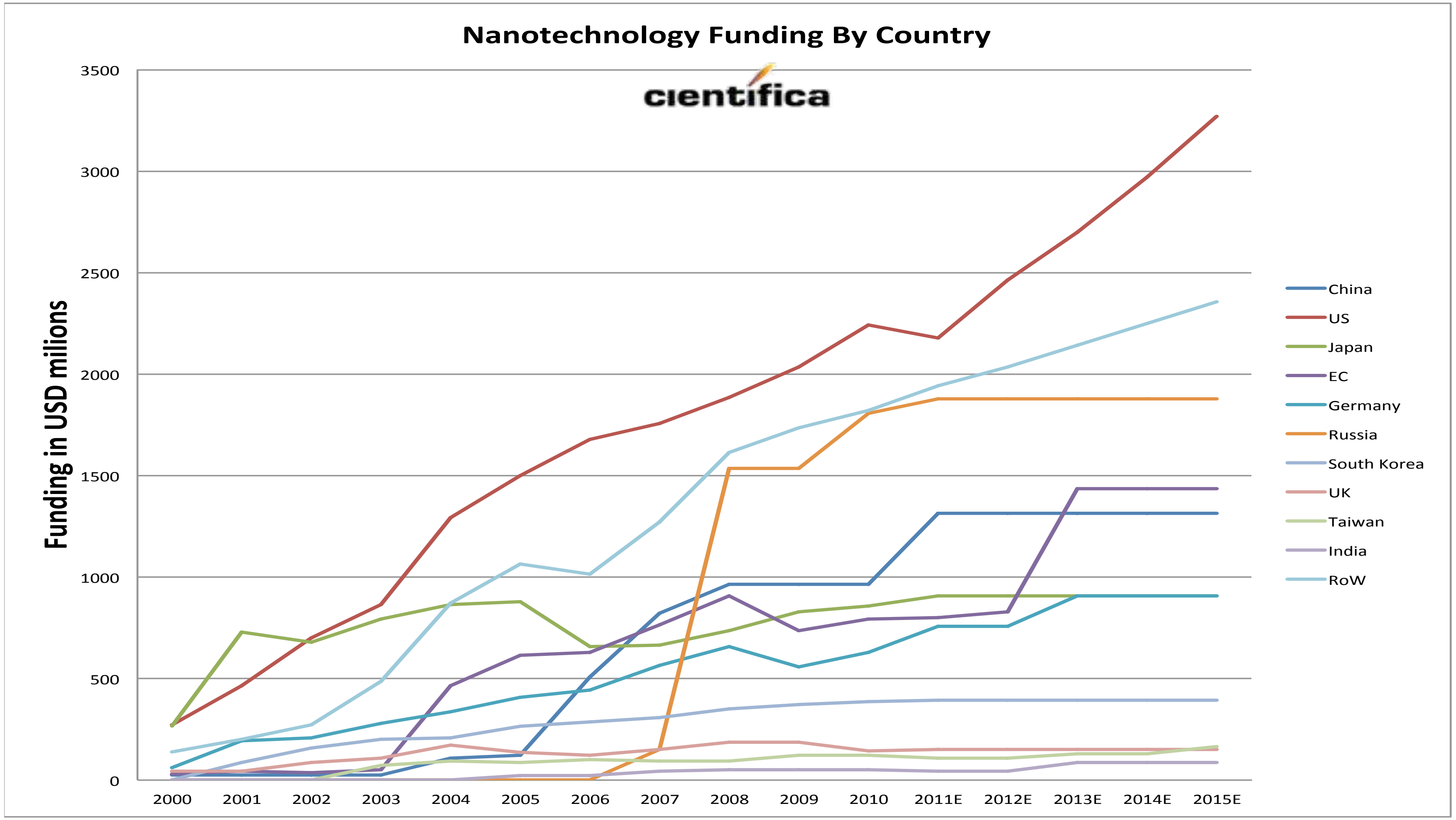
NANOGENTOOLS

- >6000 commercially available products
- >800 companies
- 47 countries
- 1 million tons / year (0.75 is ultrafine TiO_2)
- 1.5 Trillions $ by 2015 ??? (NSF, 2001)

Source 2010,http://www.nanotechproject.org/
Source 2016, http://product.statnano.com/

ENM facts

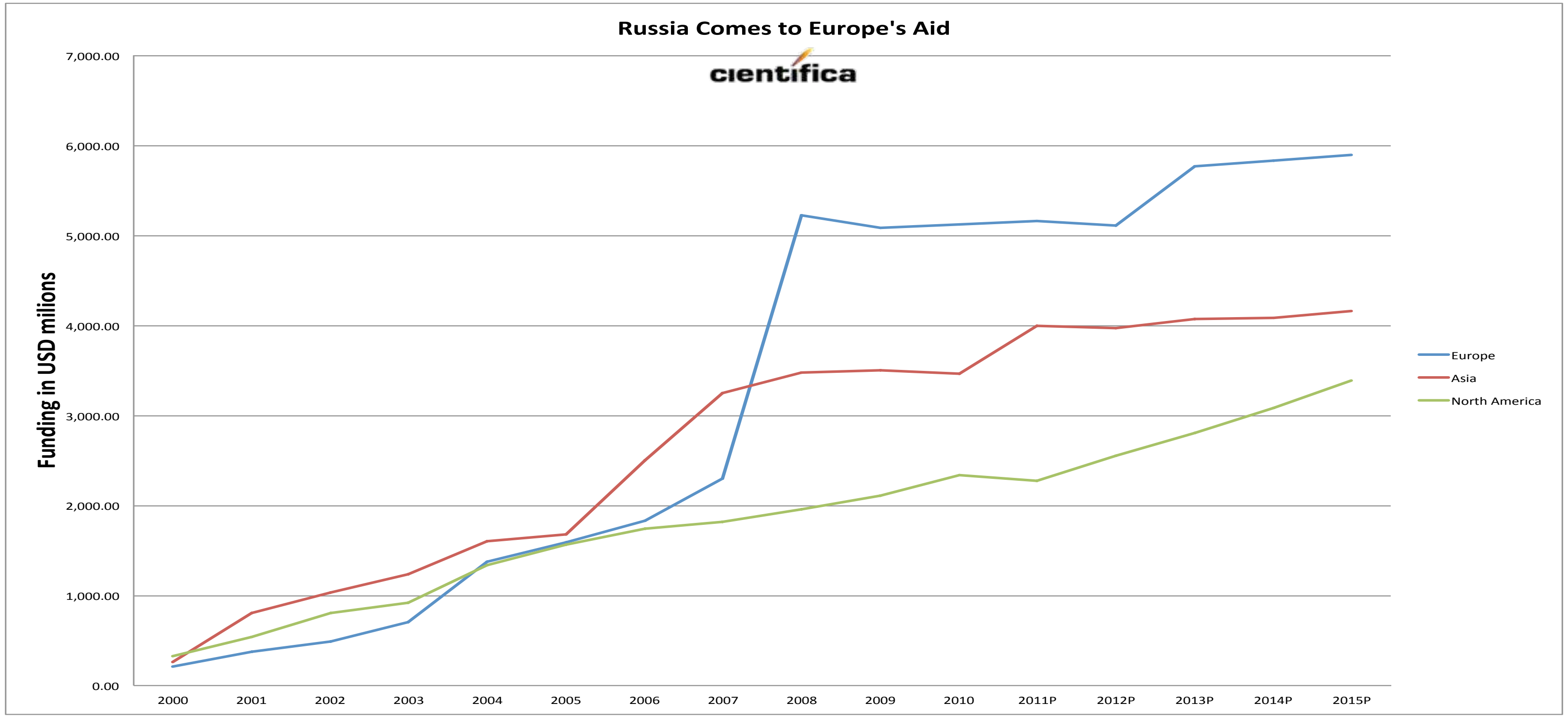
NANOGENTOOLS



Funding of nanotechnologies by country (source: Cientifica Ltd 2011)

ENM facts

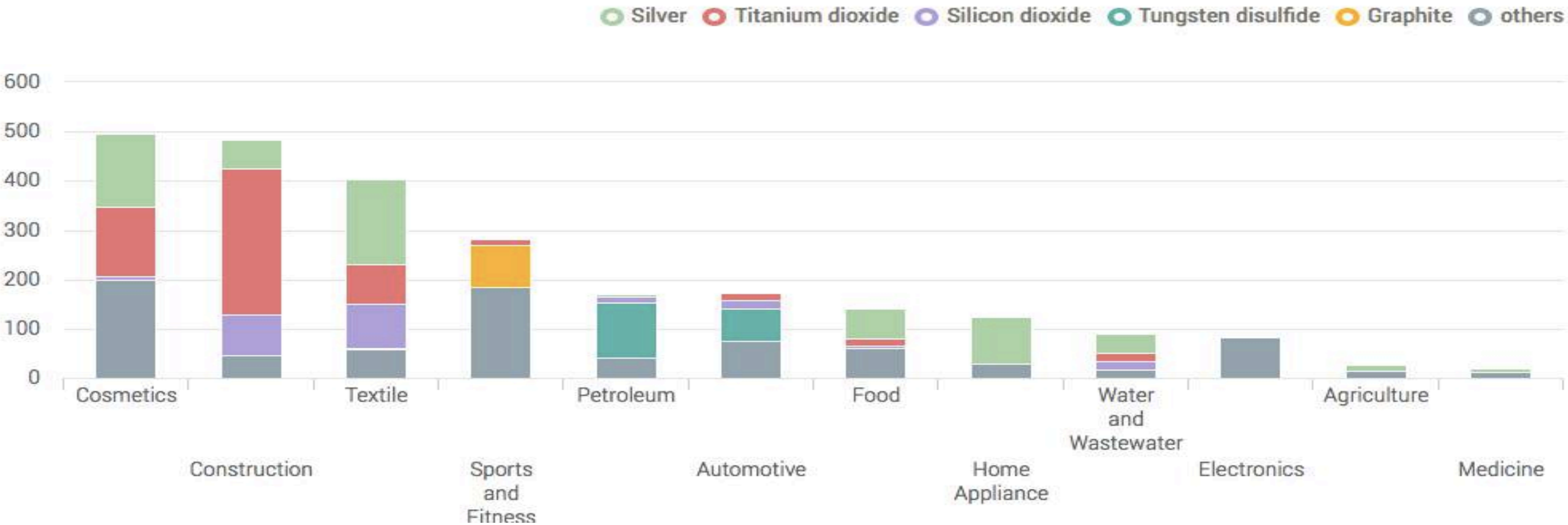
NANOGENTOOLS



Funding of nanotechnologies by region (source: Cientifica Ltd 2011)

ENM facts

NANOGENTOOLS



Source 2016, http://product.statnano.com/

Nano Silver

- Silver is an extremely reactive metal
- in rapid diffusion at European and world level
- 3 tons/y in Switzerland (one-third in the textile industry)

- Several fields of application:
  - Anti-microbial activity (food preservative, water purification, additive)
  - Paints & coating
  - Electronics
  - Industrial catalysis ?? (5000 tons/y bulk Ag)
  - …

http://www.nanotechproject.org/

AIM AND OUTLINES OF THE WORK

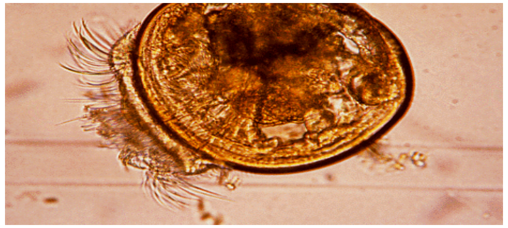
- Investigation of silver nanoparticles (AgENPs) effects in the marine environment

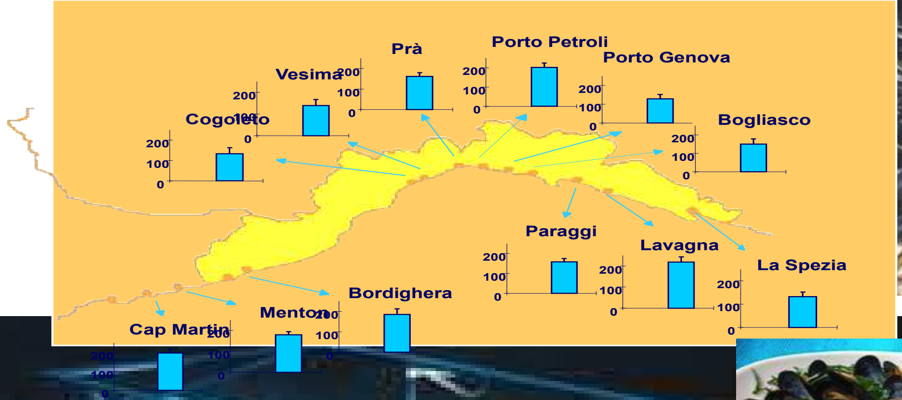
- Use of the marine filter feeding organism *Mytilus galloprovincialis* Lam as bioindicator

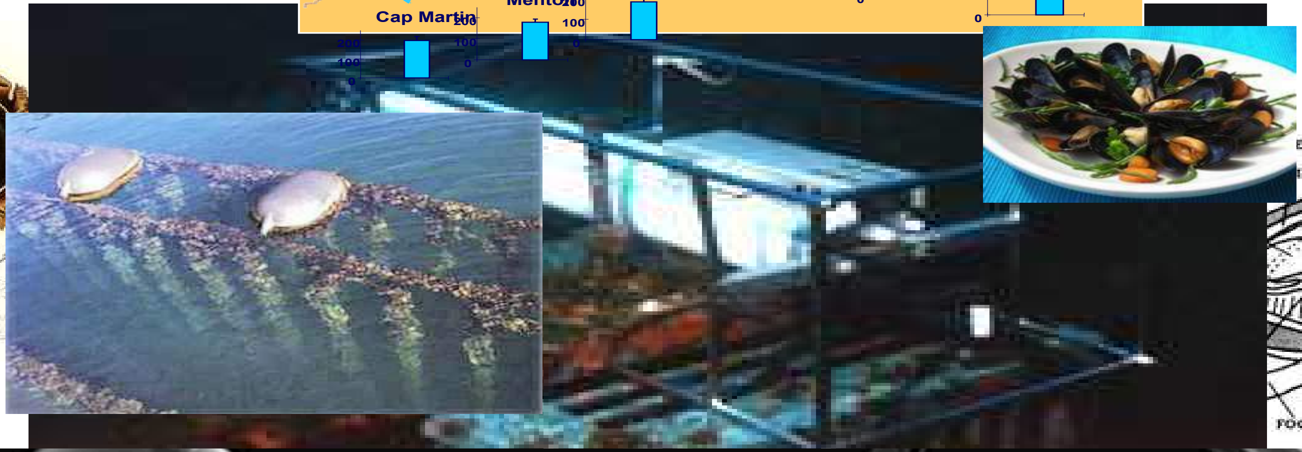
- Questions addressed:

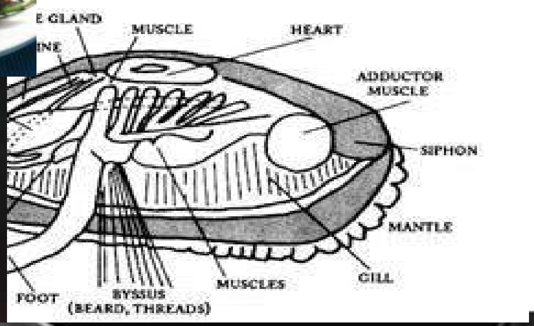
1. What is the contribution of the intrinsic **nano-form** to Ag toxicity
2. When silver NPs are toxic to mussels (Point Of Departure)?
3. Can AgENP toxicity be predicted for **high order levels** of biological organization (population)?
4. What is the **mode of action** of AgENP and what are the differences with the ionic form?

Marine mussels as env monitors









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Marine mussels as env monitors

There are four main ecotypes:
M. edulis
M. galloprovincialis
M. trossulus
M. californianus

Genetic introgression of one species into another is usual

A growing body of information on mussel physiology, genetics and genomic information is available.

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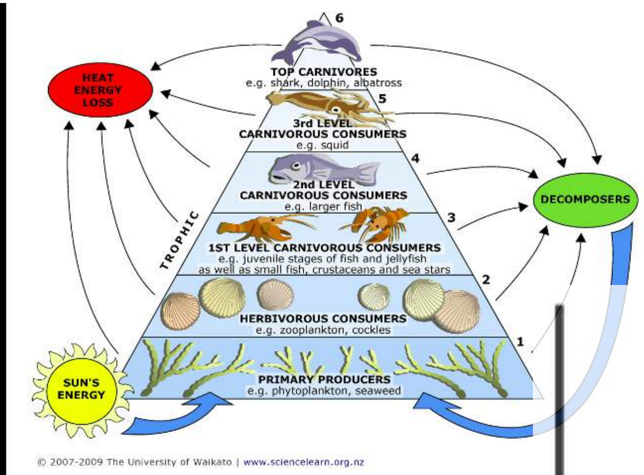
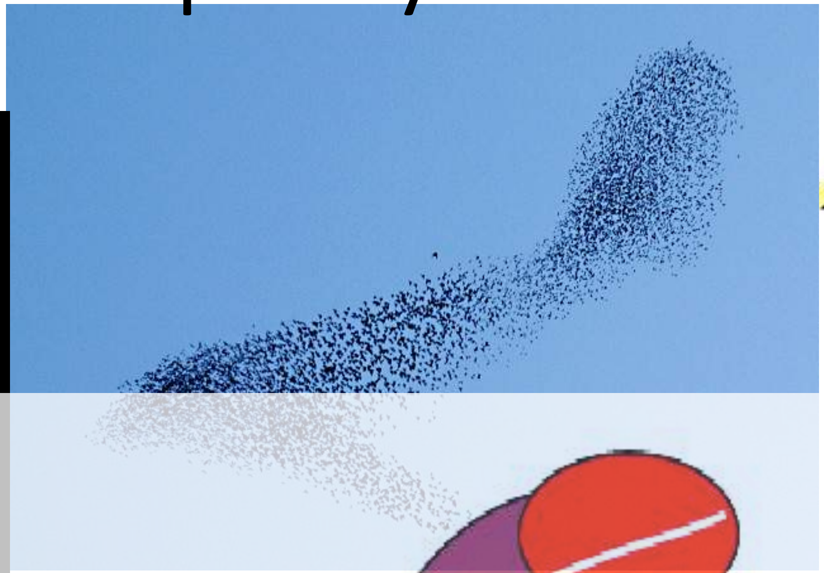
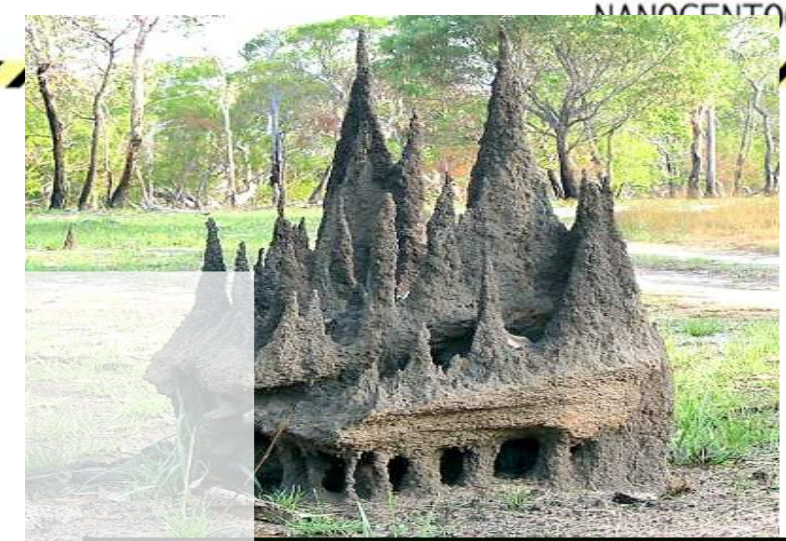
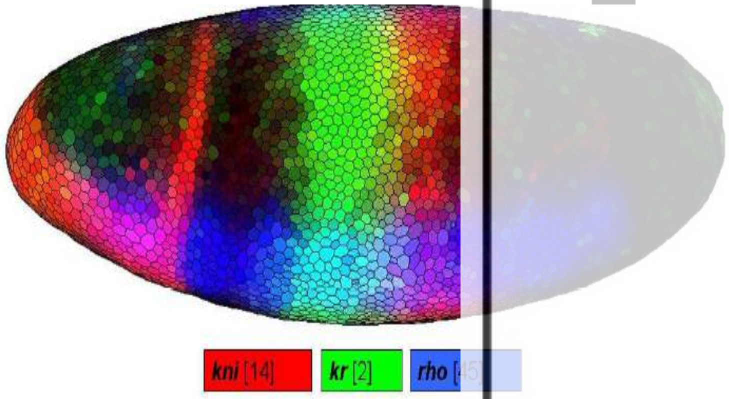
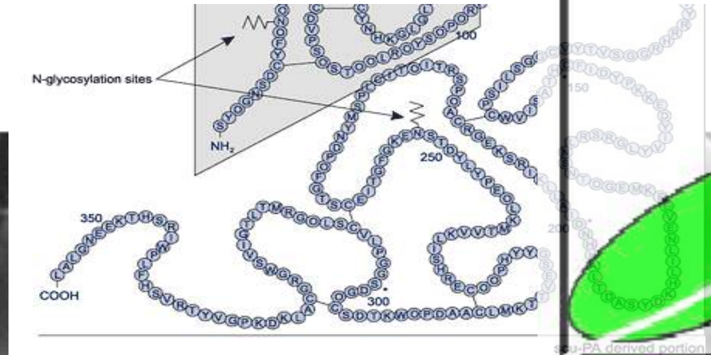
Biological complexity and informational levels

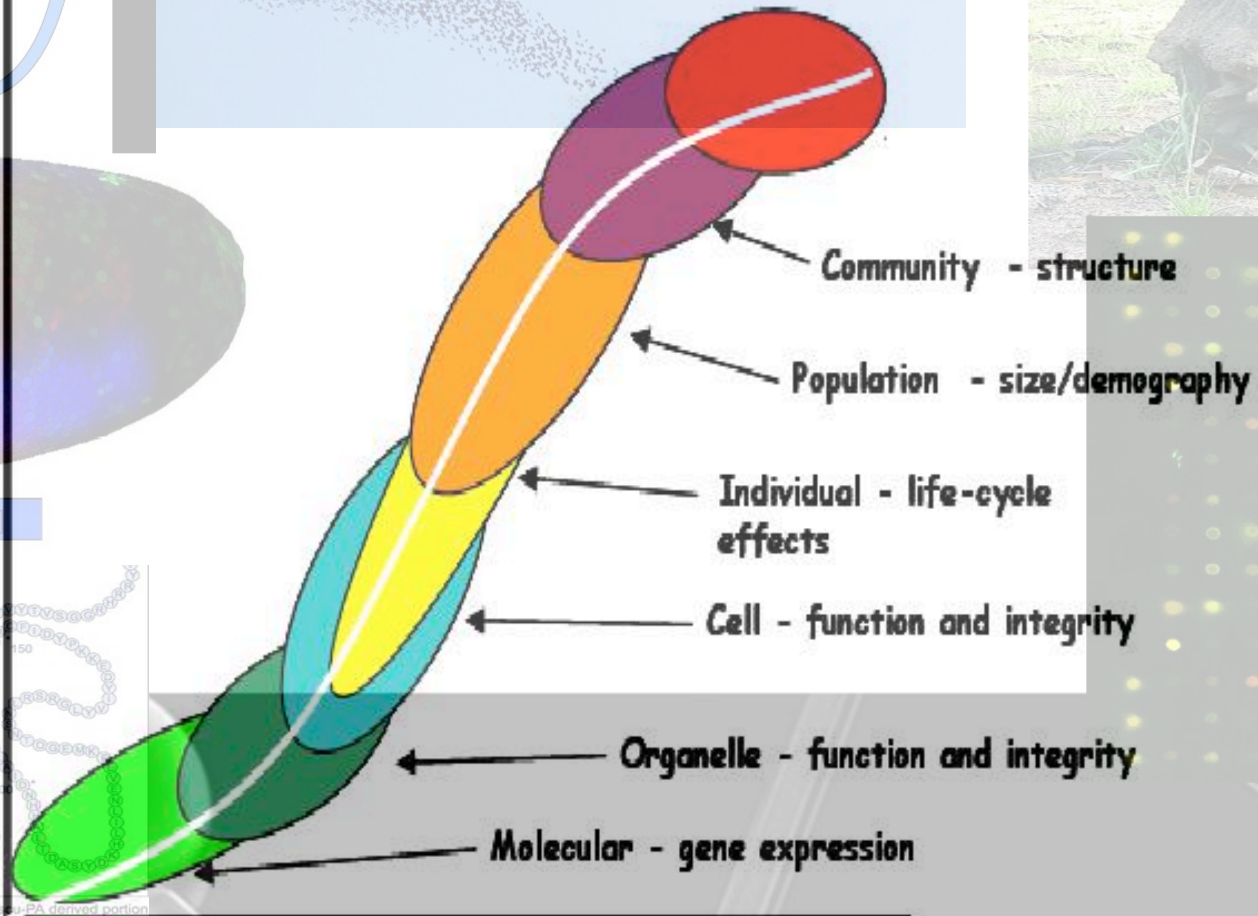


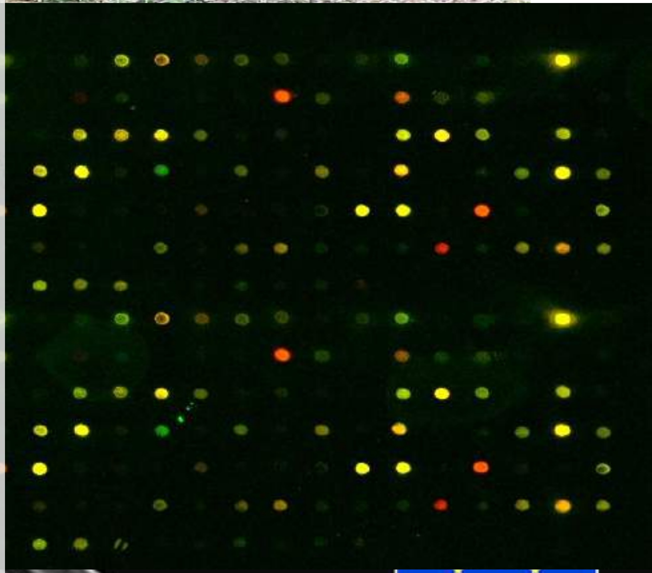












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Three levels of study:

Organismic level

toxicological descriptors and Point of Departure (POD)

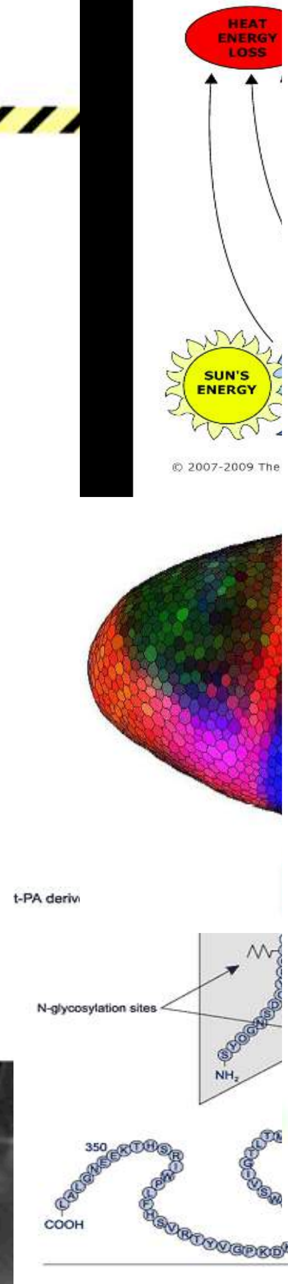
Mechanistic level

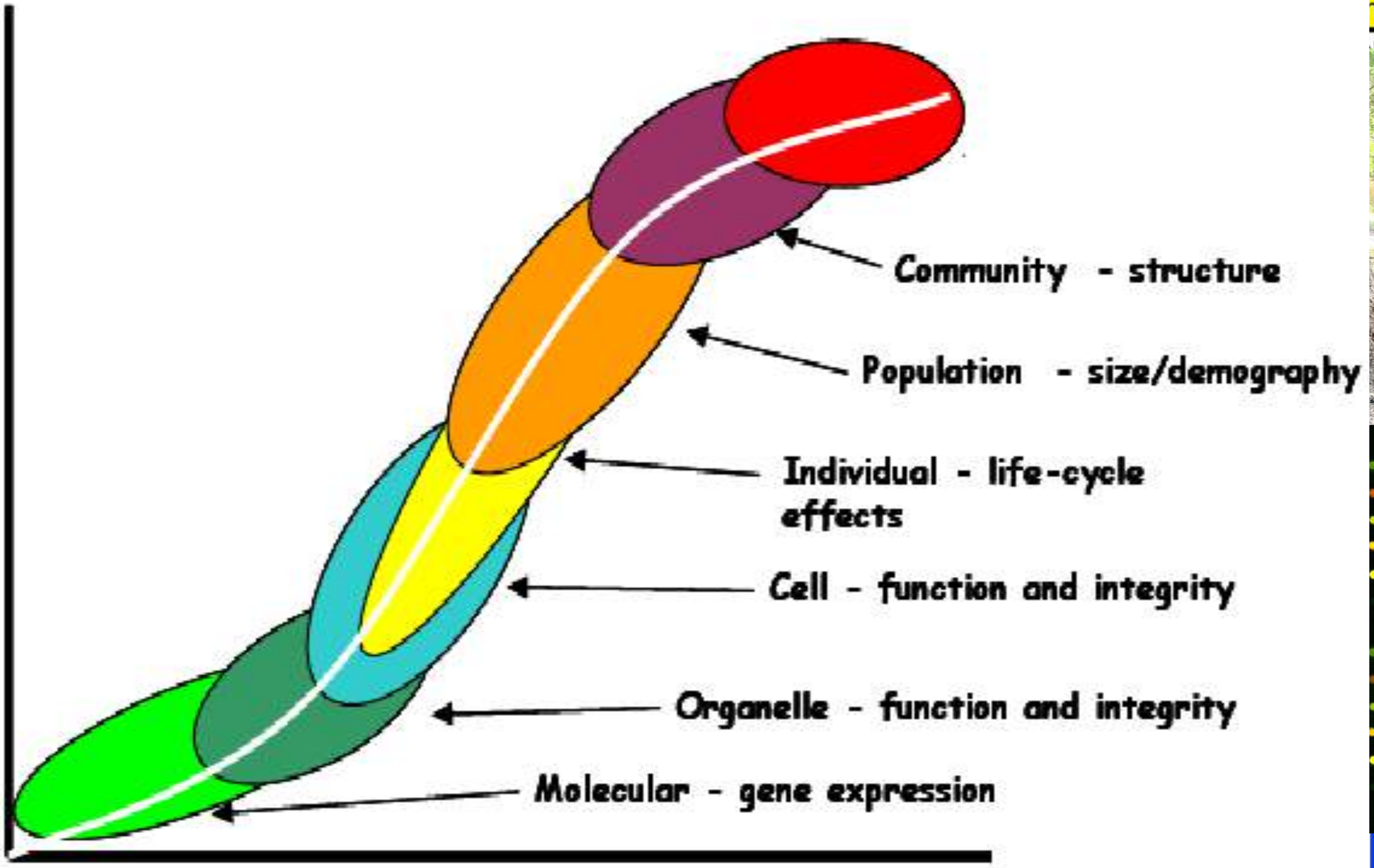
Mode Of Action (MOA) by means of a systematic assessment (**transcriptomics**)

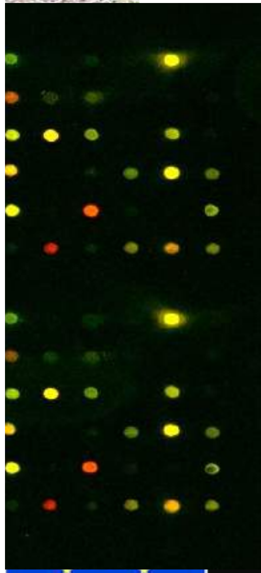
Ecophysiological level

Predictionn of high order level effects from long-term change assessment in the energy parameter responses

Biological compl[redacted]xity and informational levels



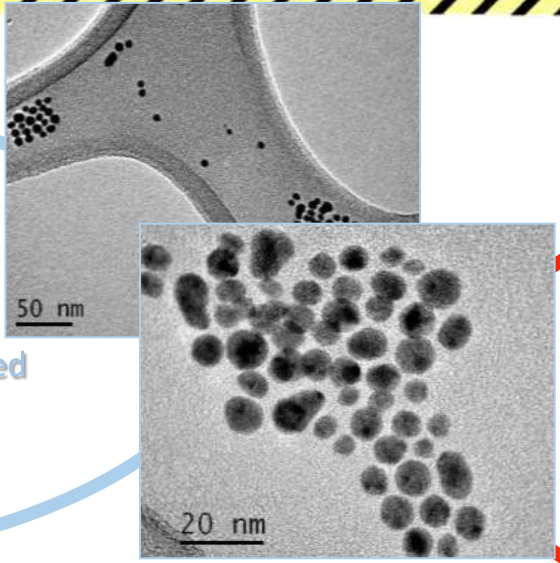




NANOPARTICLES TESTED

Ag 5nm

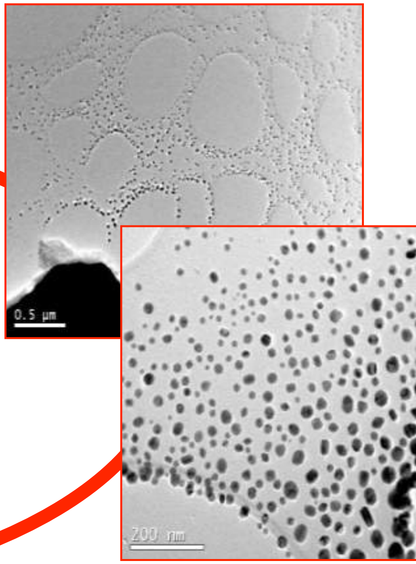
1000 ppm stable water suspension, manufactured and provided by Amepox, Poland



+

Ag 50nm

powder, manufactured by Nanotrade, Czech Republic



$AgNO_3$

Powder, Sigma Aldrich, 99%

NANOGENTOOLS

Organismic level

Ecotoxicological caracterization of ENPs (acute, subchronic and chronic tests).

Identification of full range of toxicity endpoints

Mechanistic level

Molecular, Biochemical and cytochemical approach.

Evaluation of oxidative stress by starting from Reactive Oxygen Species (ROS) based Mode Of Action (MOA)

Ecophysiological level

Evaluation of long-term changes in the energy parameters/physiological responses of bivalve.

Application of the Dynamic Energy Budget (DEB) model

EXPERIMENTAL DESIGN

Animals

Mytilus galloprovincialis in aerated 35‰ **artificial** seawater (3.5 L/min) a 18°C, pH 8 ± 0.5, 1 L per animal.
Acclimatized for 30 days (daily food addiction)

Exposure

Semi-static conditions, silver added daily along with water renewal from freshly prepared stock water-suspension.
Four days of exposure. 10 animals per biological replicate (5)

Nanoparticles, primary characterization and doses

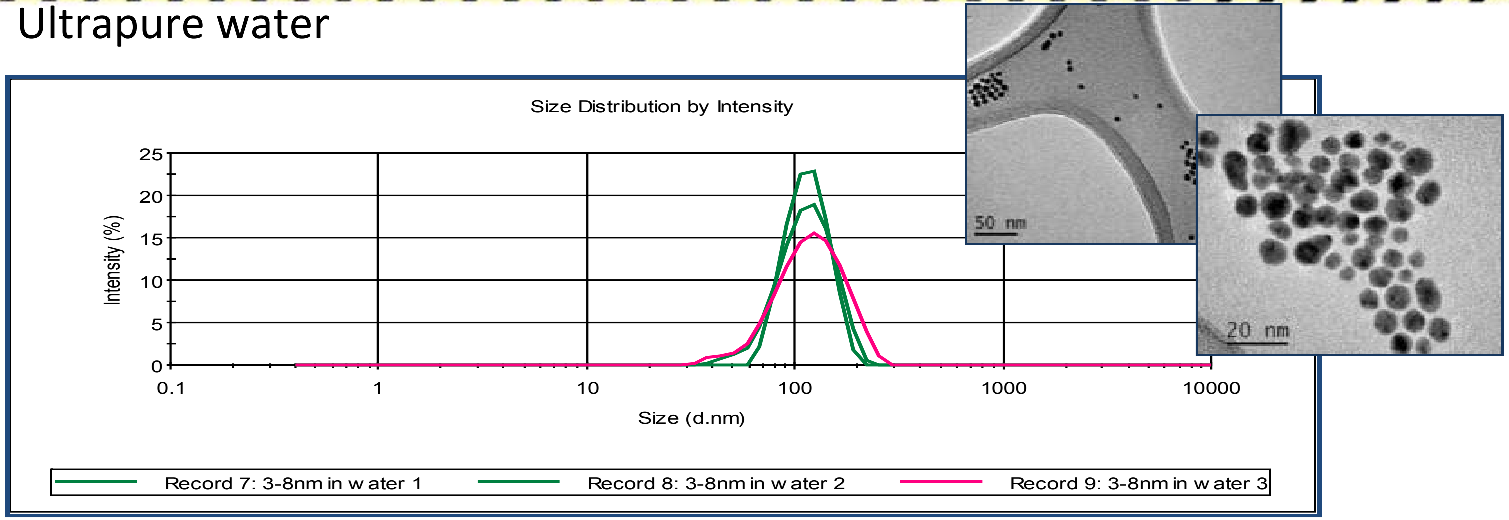
AgENPs 5nm and 50nm, $AgNO_3$
5 nominal exposure levels with a log10 series:
10 mg/L – 0.001 mg/*L*
Primary characterization of AgENPs (TEM, DLS)

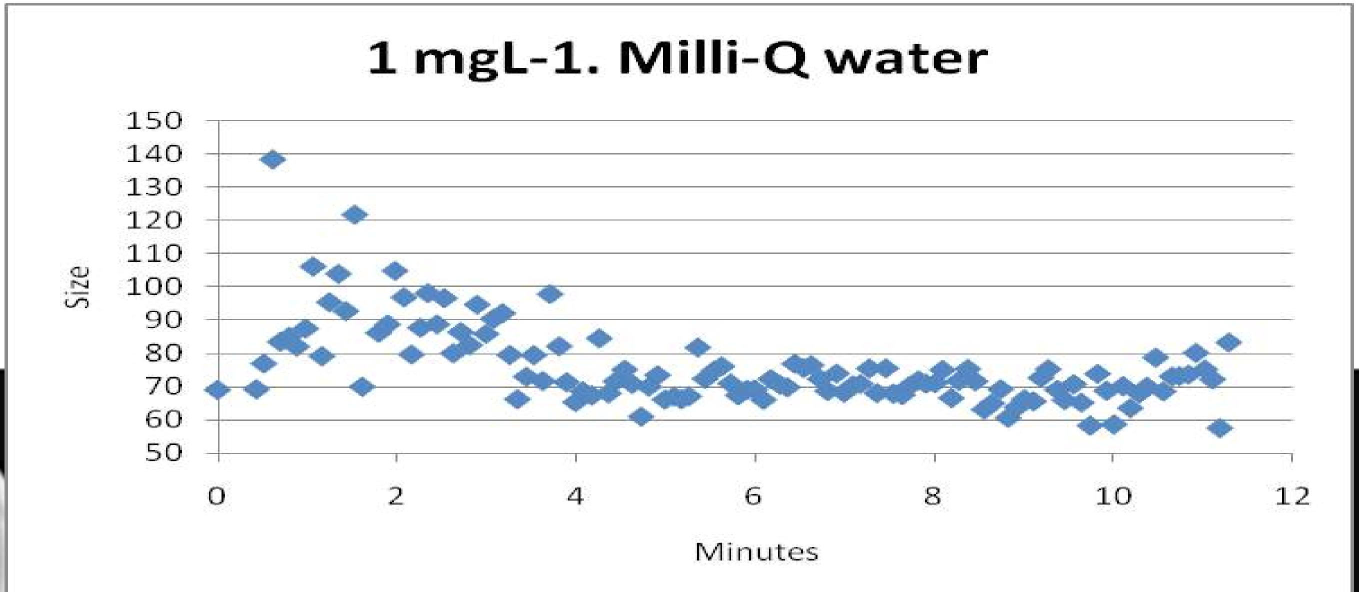
Secondary characterization

Water samples were withdrawn at regular intervals (0-1-4-24 h) and analysed for dissolved (ionic) silver fraction content (ICP-MS, LOD 0.5 µg/L).

Nano silver fate in seawater

Ultrapure water

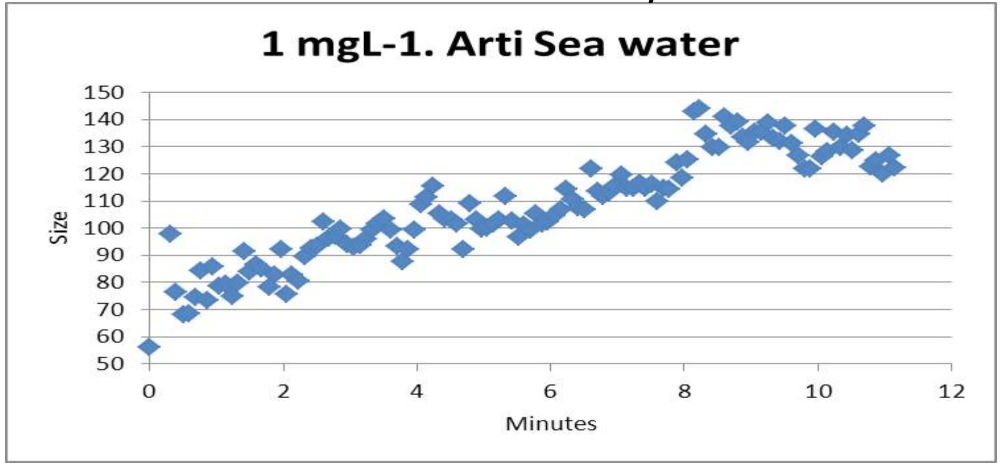




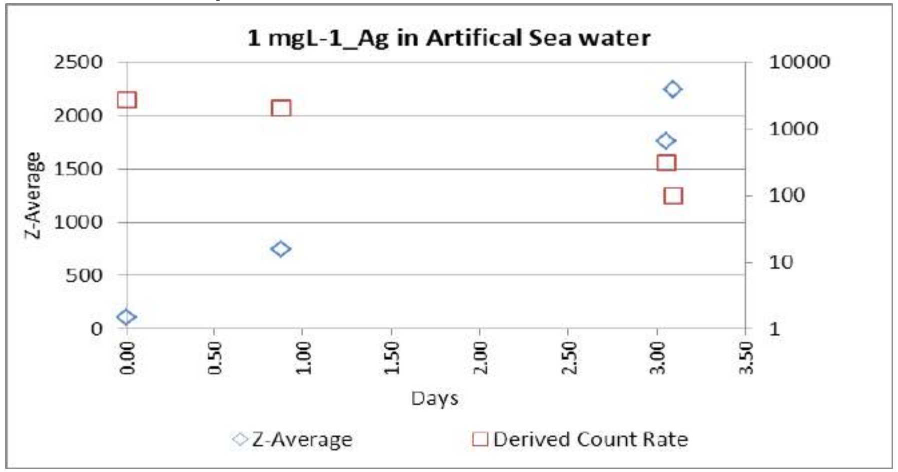
Nano silver fate

Seawater

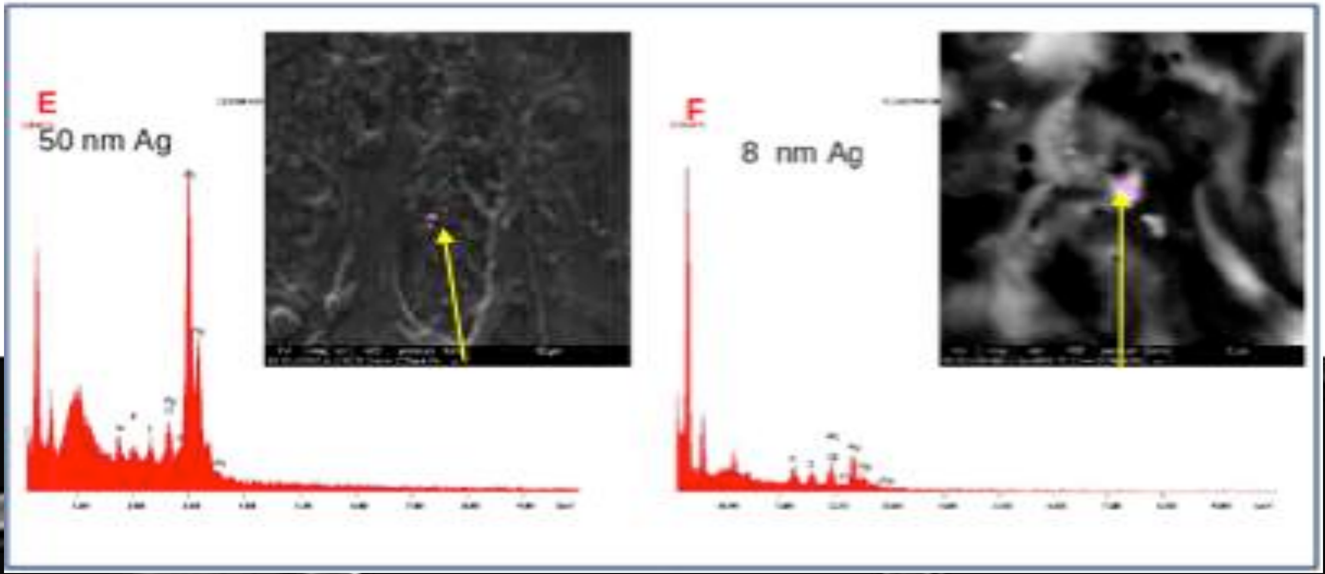
DLS analysis



DLS analysis



λESEM-EDX analysis on whole fixed gills. High dose (1 mg/L)



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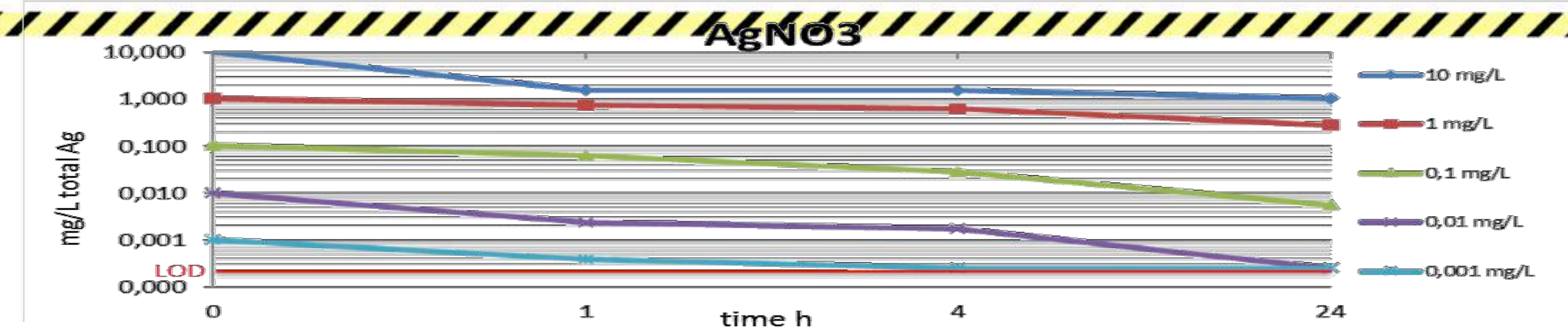
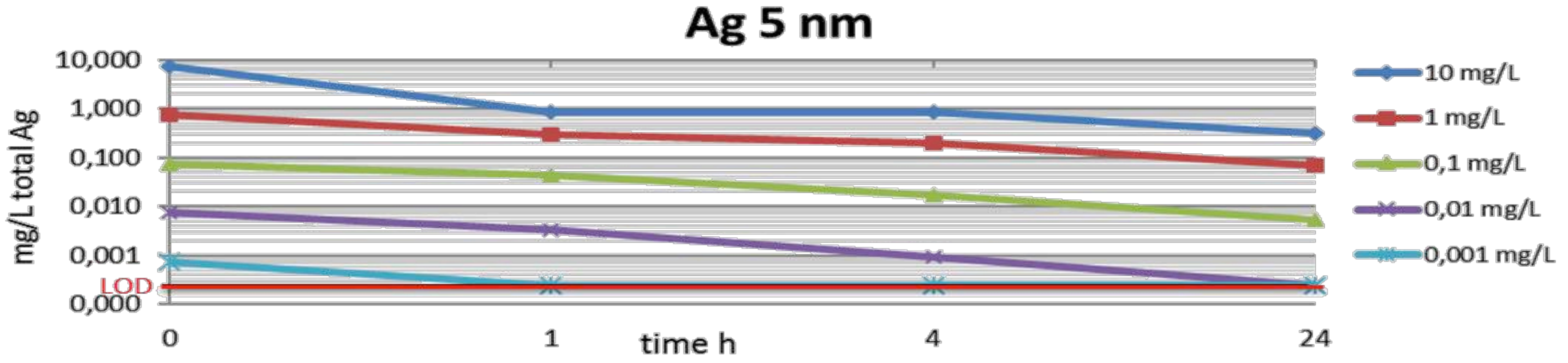
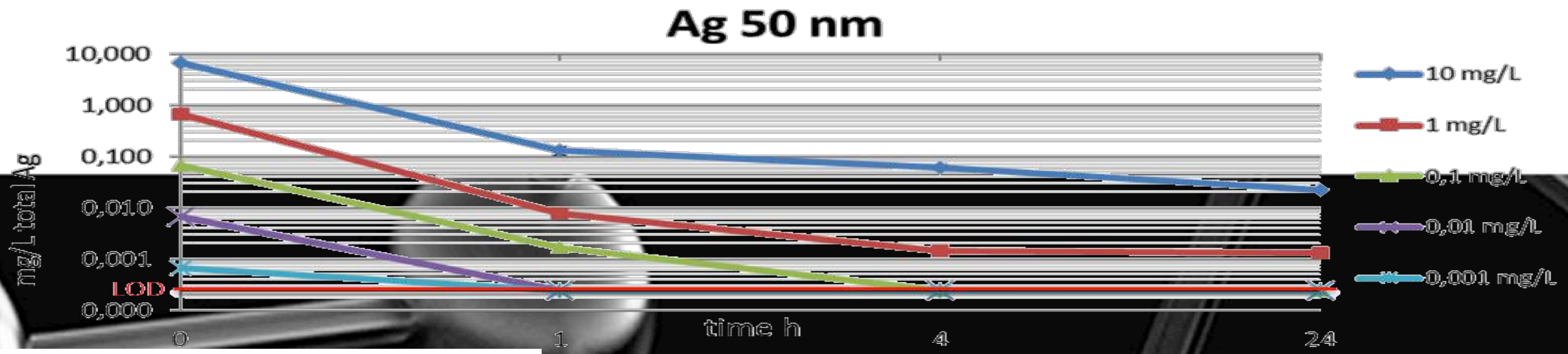
Seawater silver concentration







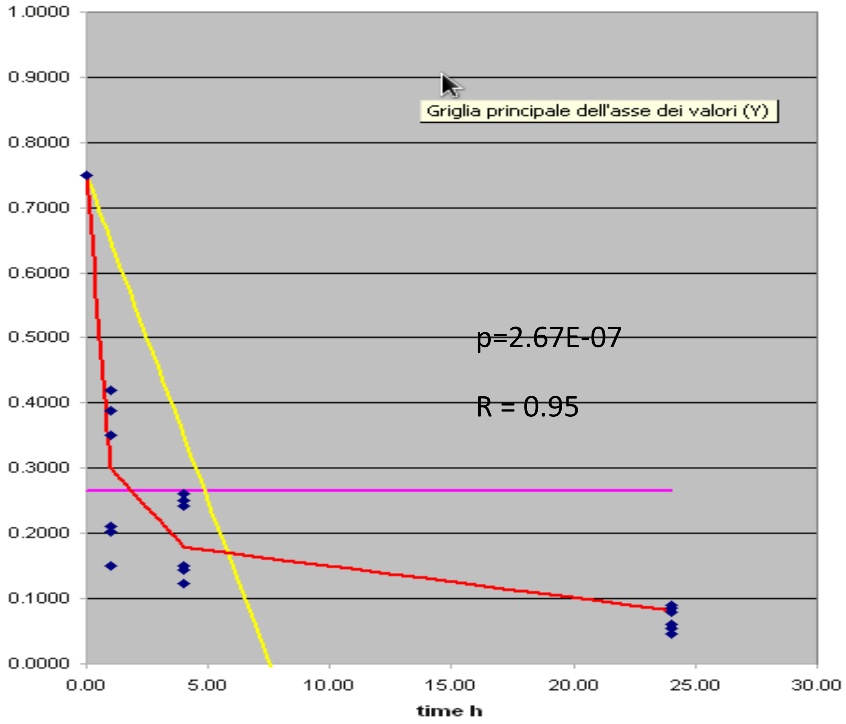
LOD (detection limit of the technique): 0,0005 mg/L

A kinetic model for silver availability

- Set up kinetic models for silver bioavailability in the water column based on the logistic curve (starting from ICP-MS data of total silver for each Ag form)

	differenza	SS	Model	SS		Model	SS
9	0.4840	0.234256	0.75	0		0.75	0
10	-0.0640	0.004096	0.65	0.200704		0.300923	0.009785817
11	0.1230	0.015129	0.65	0.068121		0.300923	0.007757507
12	-0.1160	0.013456	0.65	0.25		0.300923	0.022777839
13	0.0840	0.007056	0.65	0.09		0.300923	0.002408524
14	-0.0560	0.003136	0.65	0.1936		0.300923	0.008267045
15	0.1540	0.023716	0.65	0.0529		0.300923	0.014179263
16	-0.1220	0.014884	0.35	0.042436		0.180347	0.001321096
17	-0.0240	0.000576	0.35	0.011664		0.180347	0.003801106
18	-0.1160	0.013456	0.35	0.04		0.180347	0.000920934
19	-0.0160	0.000256	0.35	0.01		0.180347	0.004851556
20	-0.1430	0.020449	0.35	0.051529		0.180347	0.003288666
21	-0.0060	3.6E-05	0.35	0.0081		0.180347	0.006344618
22	-0.2060	0.042436	-1.65	2.9241		0.080429	0.00041736
23	-0.1862	0.03467044	-1.65	2.99220804		0.080429	3.96123E-07
24	-0.2110	0.044521	-1.65	2.907025		0.080429	0.000646654
25	-0.1810	0.032761	-1.65	3.010225		0.080429	2.08905E-05
26	-0.2210	0.048841	-1.65	2.873025		0.080429	0.001255241
27	-0.1760	0.030976	-1.65	3.0276		0.080429	9.15967E-05

Example: 5 nm Ag-NP kinetic bioavailability model (1 mg)



NANOGENTOOLS

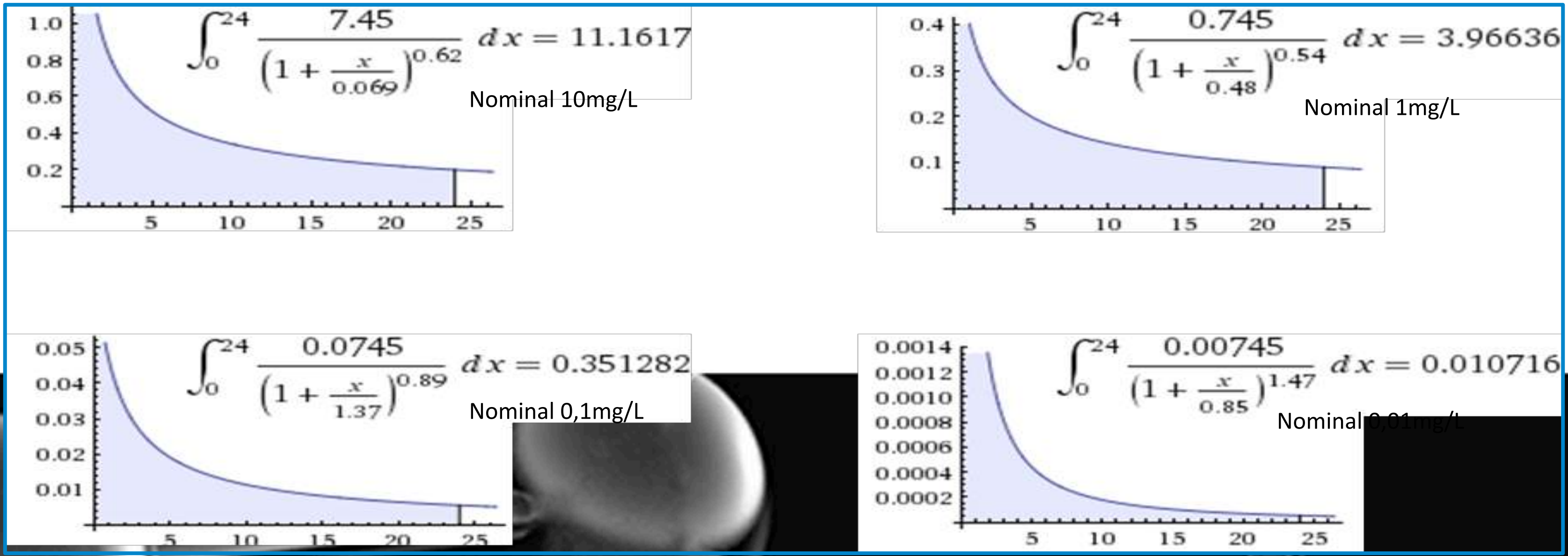
INTEGRATED STANDARDIZED SILVER DOSE

$$y = max/(1+(x/\tau))^b$$

y = Silver concentration (mg/L)
x = time (h)
τ = half life (h) of silver in the water column

b = slope of the log phase
max = silver concentration at time zero

Determination of ACTUAL SILVER DOSE



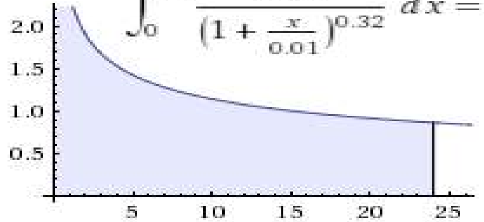
Actual silver doses for 5 nm AgENP (mg/L/d)

A kinetic model for silver availability

Silver Nitrate

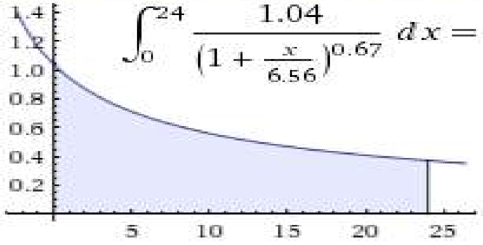
1

$$\int_0^{24} \frac{10.4}{(1+\frac{x}{0.01})^{0.32}}\,dx = 30.2693$$



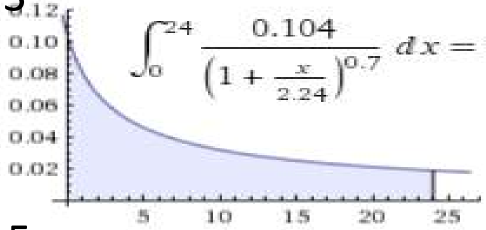
2

$$\int_0^{24} \frac{1.04}{(1+\frac{x}{6.56})^{0.67}}\,dx = 13.6776$$



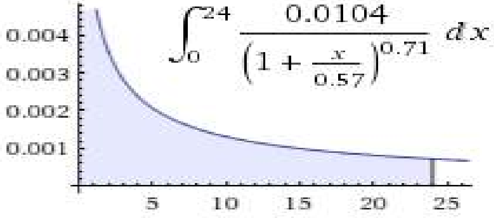
3

$$\int_0^{24} \frac{0.104}{\left(1+\frac{x}{2.24}\right)^{0.7}}\,dx = 0.848173$$



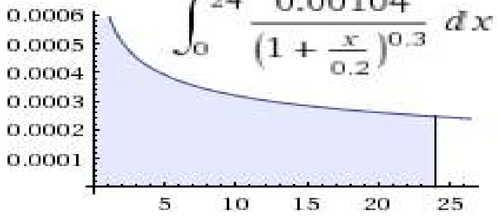
4

$$\int_0^{24} \frac{0.0104}{\left(1+\frac{x}{0.57}\right)^{0.71}}\,dx = 0.0404455$$



5

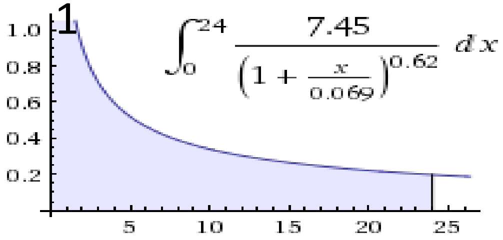
$$\int_0^{24} \frac{0.00104}{(1+\frac{x}{0.2})^{0.3}}\,dx = 0.00823219$$



Small Ag ENPs

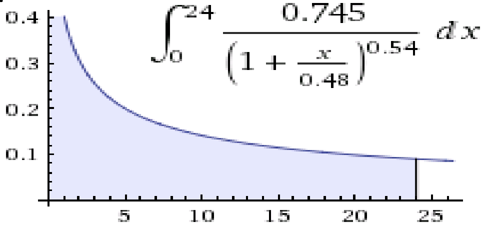
1

$$\int_0^{24} \frac{7.45}{\left(1+\frac{x}{0.069}\right)^{0.62}}\,dx = 11.1617$$



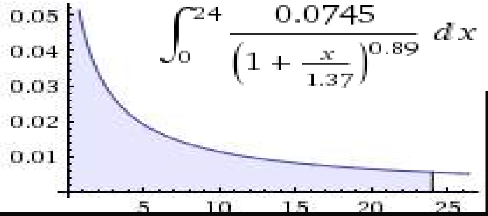
2

$$\int_0^{24} \frac{0.745}{\left(1+\frac{x}{0.48}\right)^{0.54}}\,dx = 3.96636$$



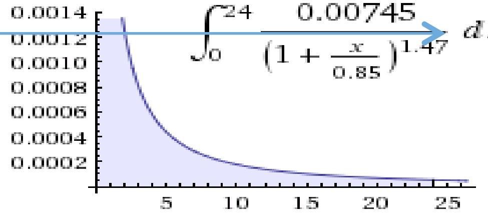
3

$$\int_0^{24} \frac{0.0745}{\left(1+\frac{x}{1.37}\right)^{0.89}}\,dx = 0.351282$$



4

$$\int_0^{24} \frac{0.00745}{\left(1+\frac{x}{0.85}\right)^{1.47}}\,dx = 0.010716$$



Acute toxicity as a function of actual silver dose (96h)

Organismic level

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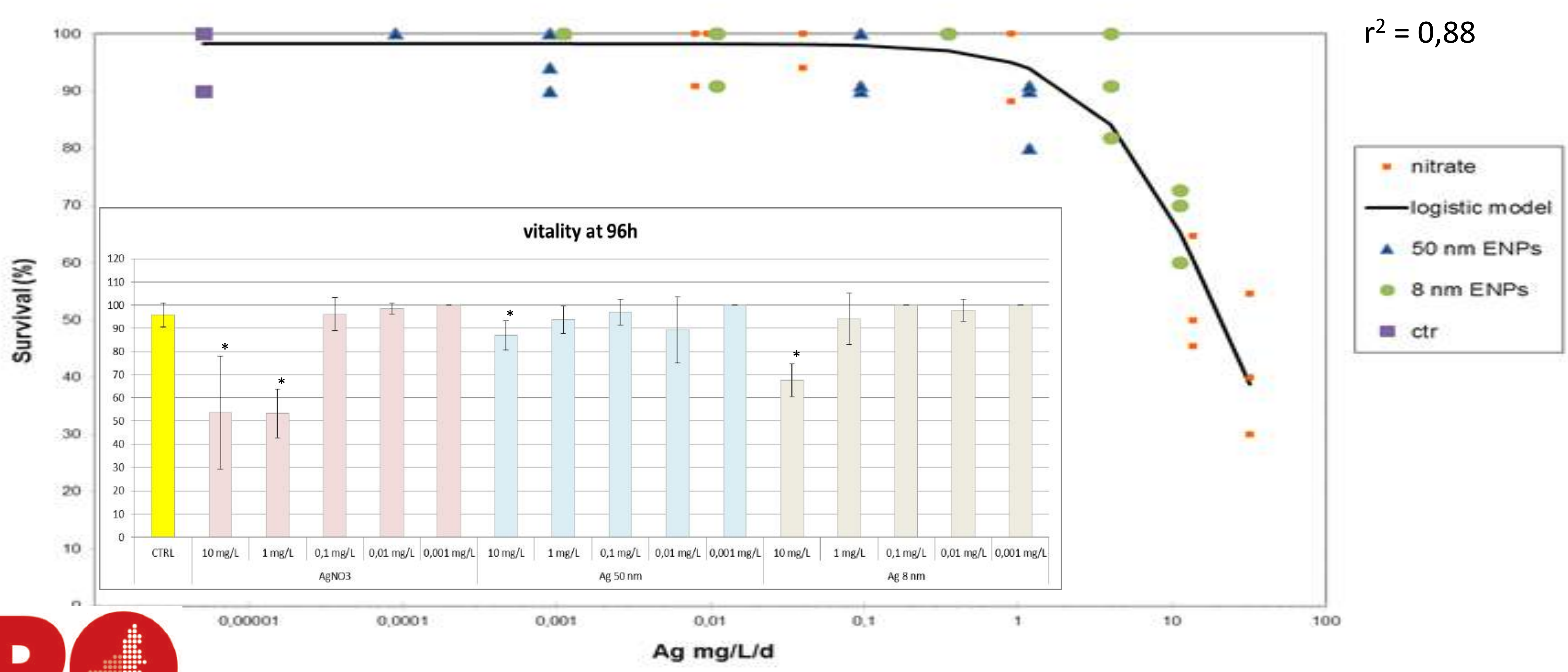

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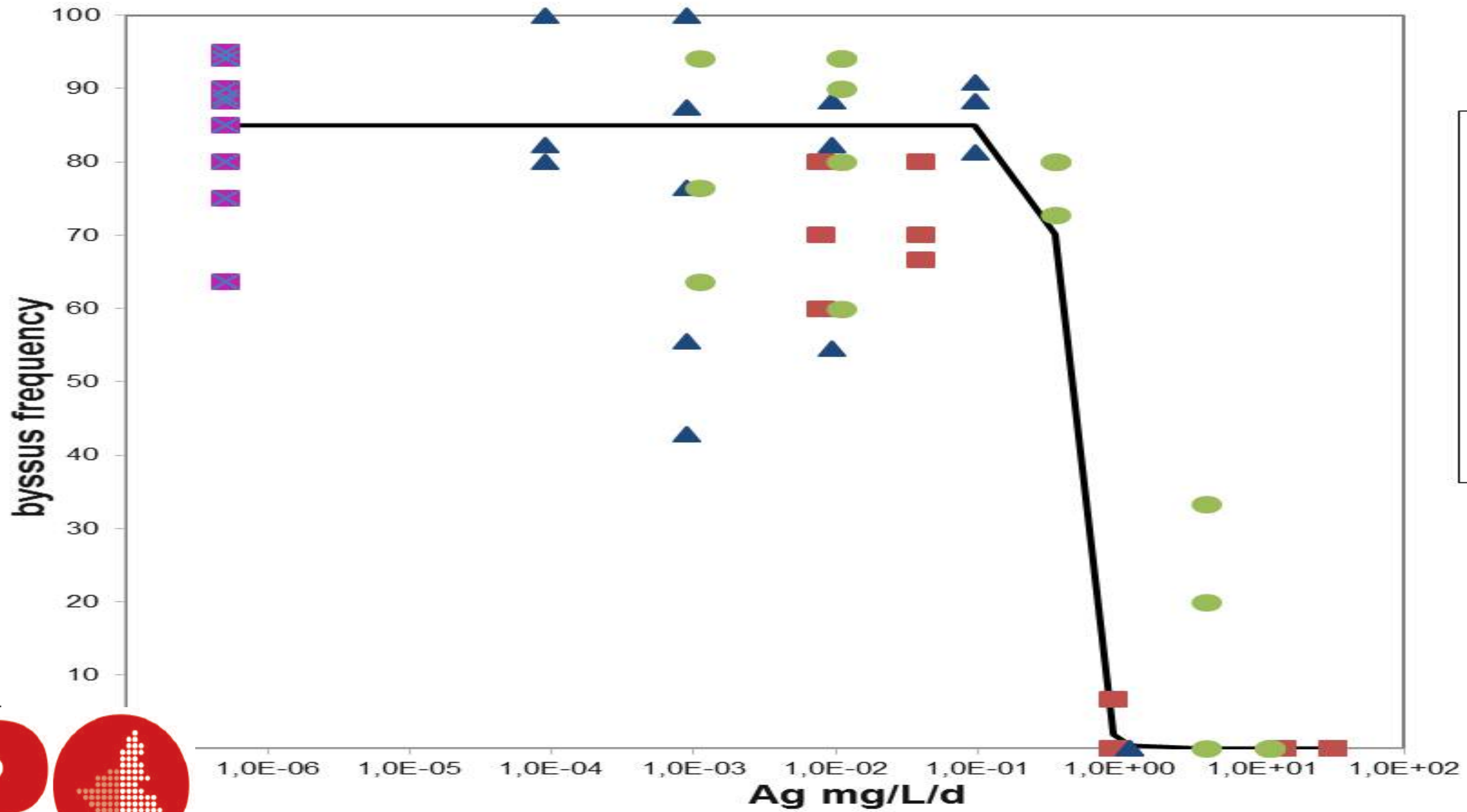
Organismic level

Chronic toxicity (byssus) as a function of actual silver dose

NANOGENTOOLS

$r^2 = 0{,}79$



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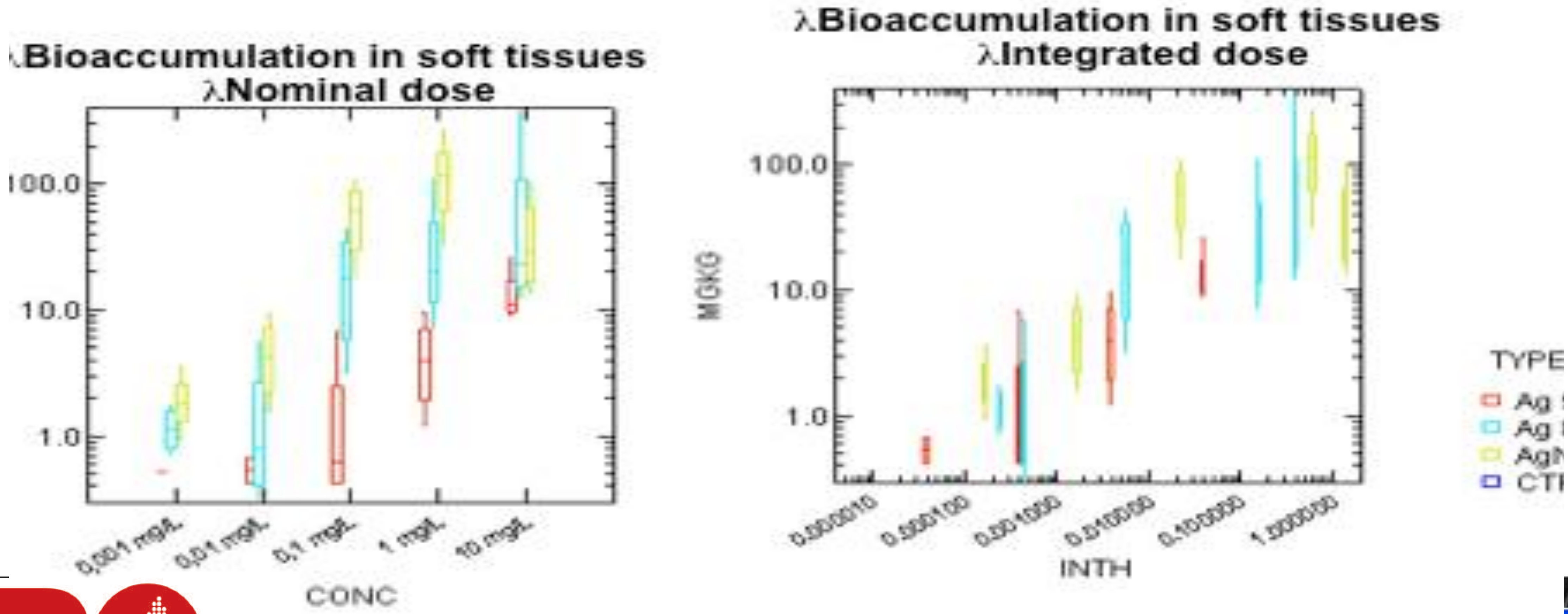
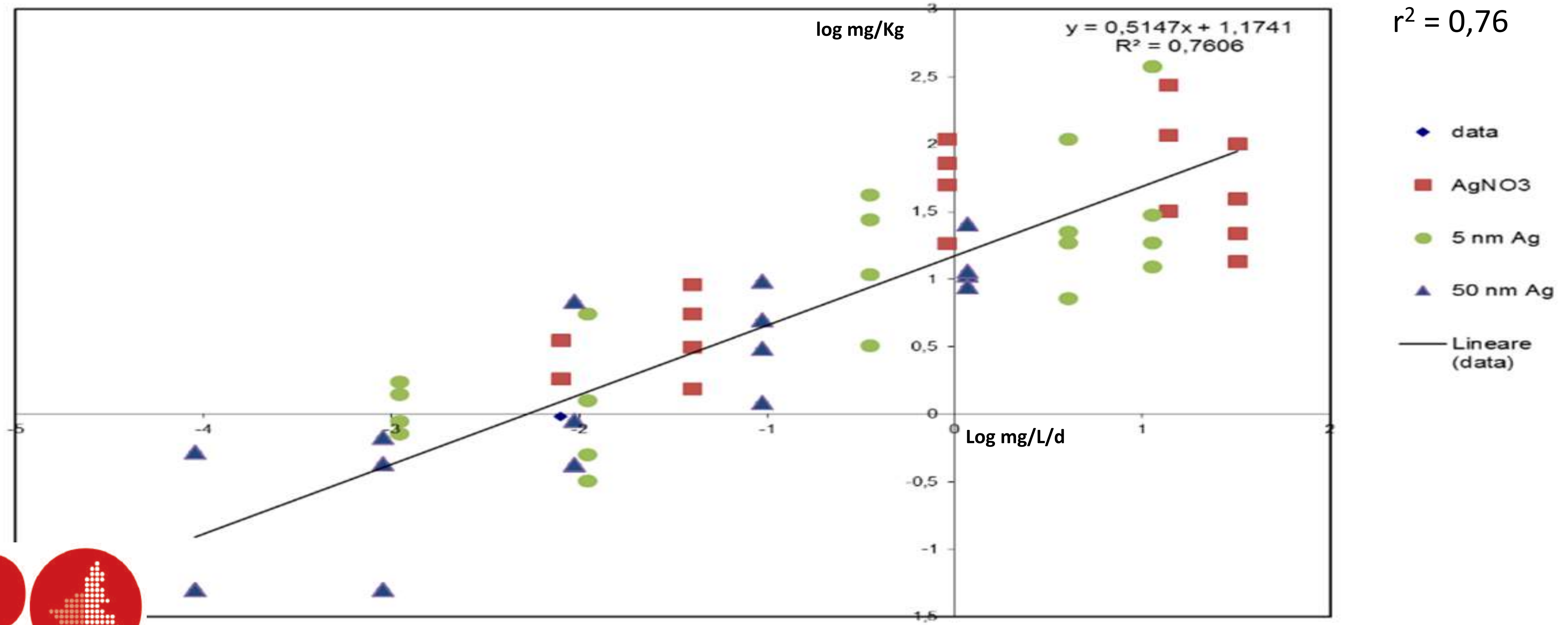
Bioaccumulation patters



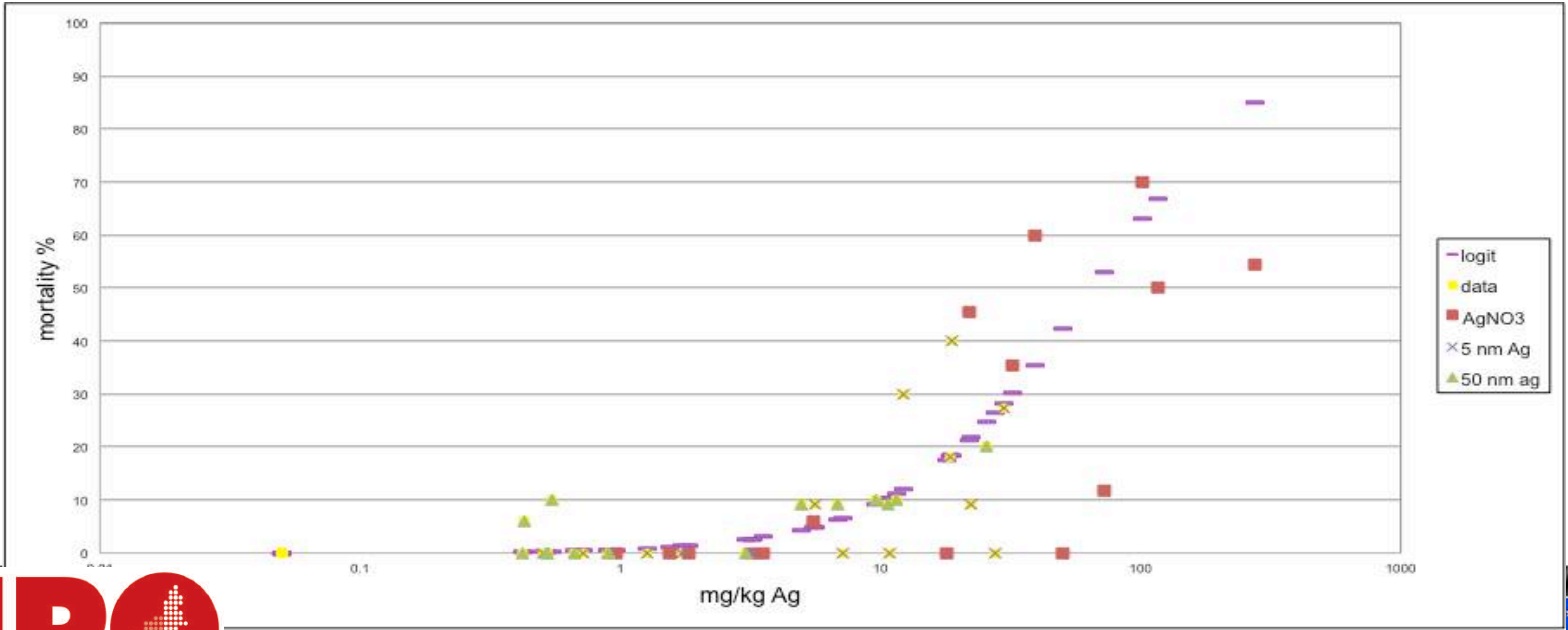
Bioaccumulation of silver in mussel body as a function of actual silver dose (log-log)



$r^2 = 0,76$

NANOGENTOOLS

Mortality vs internal dose



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NANOGENTOOLS

Take home message

- *The main factor driving gross silver toxicity, i.e. mortality is the **actual** concentration in the water column;*

- *The "particle effect" is relevant in the context of chemical/ physical processes occurring in the seawater (sedimentation, aggregation, etc)*

Organismic level

NANOGENTOOLS

ECOTOXICOLOGICAL ENDPOINTS

particle size/other characteristic	endpoint	NOEC	LOEC	EC1	EC5	EC10	EC20	EC50	units
				Regressed value					
$AgNO_3$	96h mortality	0,02083	0,56958	0,00176	0,01565	0,04203	0,12279	0,76764	mg/L/h
amepox 3-8nm	96h mortality	0,16625	0,40667	0,00802	0,04089	0,08546	0,19022	0,74697	mg/L/h
nanotrade 50 nm	96h mortality	0,00445	0,04454	0,00035	0,00618	0,02271	0,09332	1,04492	mg/L/h
$AgNO_3$	survival time probability after emersion	0,00156	0,02083	na		na	na	na	mg/L/h
amepox 3-8nm	survival time probability after emersion	0,00046	0,00596	na		na	na	na	mg/L/h
nanotrade 50 nm	survival time probability after emersion	0,00004	0,00045	na		na	na	na	mg/L/h
$AgNO_3$	bissus synthesis	0,00156	0,02083	0,00039	0,00090	0,00130	0,00195	0,00388	mg/L/h
amepox 3-8nm	bissus synthesis	0,00596	0,16625	0,00007	0,00056	0,00146	0,00415	0,02464	mg/L/h
nanotrade 50 nm	bissus synthesis	0,00445	0,04454	na	na	na	na	na	mg/L/h

5 nm AgENPs and $AgNO_3$ were tested

TWO nominal exposure concentrations were considered **according to the Ag logistic model**:

Actual dose (silver standardized integrated dose)

- ≅ **1.0 µg/L/h (5 nm AgENP EC_{10} (LOEC) for short term chronic toxicity test)**
- ≅ **0.1 µg/L /h (5 nm AgENP EC_1 (NOEC) for short term chronic toxicity test**)

Nominal dose

- 20 µg/L
- 2.0 µg/L

5 nm AgENPs and **$AgNO_3$** were tested

SAME **ACTUAL** RANGE FOR SILVER NITRATE WAS SELECTED

Nominal dose	Actual dose (silver standardized integrated dose)	
0.2 µg/L	**--> 0.1 µg/L/h**	**comparable with AgNP2**
2 µg/L	***--> 1 µg/L /h***	***comparable with AgNP20***
20 µg/L	--> 10 µ/L/h	10 times higher than any AgNP

NANOGENTOOLS

AgENPs impacts on physiological performance of *Mytilus galloprovincialis* Lam.

Animals were kept in **mesocosms (microcosms)** for **4 weeks.**

Acclimatation: animals transported to the laboratory under temperature/humidity controlled conditions, cleaned from epibionts and allowed to acclimate for 15 days at a temperature of 22 °C (natural filtered seawater ~37 ‰ salinity; pH 8.0-8.1; constantly aerated 60 L/h). Organisms **were fed daily** fresh cultures of *Nannochloropsis spp.* or *Isochrysis galbana* using adjustable drip.

Experimental design: Organisms treated for 28 days in mesocosms. Ag added daily to experimental samples. Natural filtered seawater (1 L/animal) constantly aerated at 60 L/h was used. Organisms were fed ad libitum with fresh algal cells of *Nannochloropsis spp* or *Isochrysis galbana.* Temperature, pH and [0_2] were daily monitored and maintained at constant level (25°C and pH value of 8.0 ± 0.2)

Organismic level

Ecotoxicological caracterization of ENPs (acute, subchronic and chronic tests).

Identification of full range of toxicity endpoints

Mechanistic level

Molecular, Biochemical and cytochemical approach.

Evaluation of oxidative stress by starting from Reactive Oxygen Species (ROS) based Mode Of Action (MOA)

Ecophysiological level

Evaluation of long-term changes in the energy parameters/physiological responses of bivalve.

Application of the Dynamic Energy Budget (DEB) model

Clearance rate (CR)

- Filtration efficiency measured as filtration capacity of food by mussels in a defined volume of water within two hours

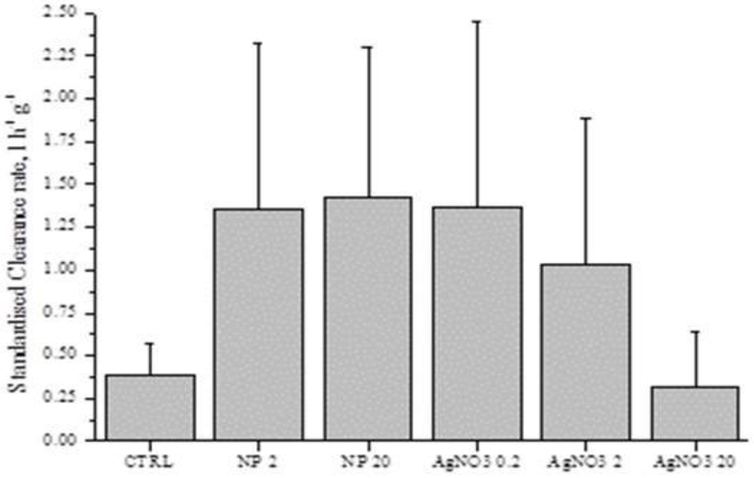
Absorption Efficiency (AE)

- Efficiency with which organic matter is absorbed by mussels from the ingested food

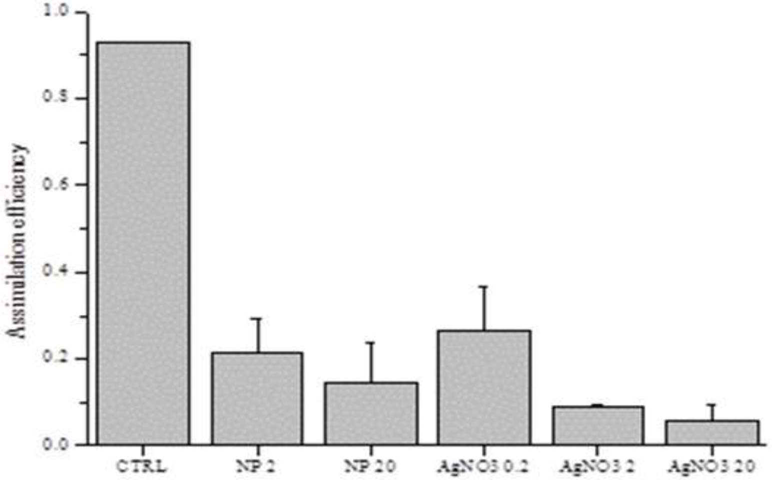
Respiration rate (RR)

- Measurement of the respiration rate of individuals through sensor arranged for measuring the decline in dissolved oxygen within a respirometric chamber

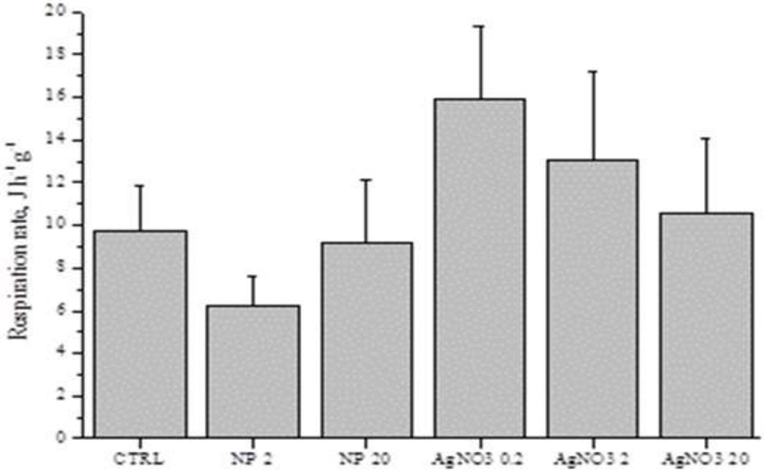
Ecophysiological effects



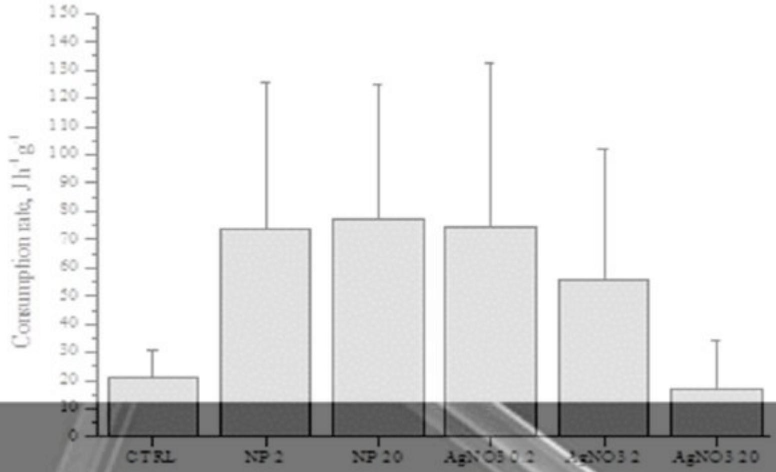
Clearance rate
CR = (Vol) × (loge C1 − loge C2) / time interval in h



Absorption efficiency
AE = (F-E)/[(1-E) F]



Respiration rate
RR = [C(t0) – (C(t1)] · (Vr) · 60/(t1 – t0)



Consumption rate
C = FR (L h^{-1} g^{-1} DW) x OM (mg L^{-1}) x 0.5 (mg C mg^{-1} OM) x 18.43 J mg^{-1}

NANOGENTOOLS

SCOPE FOR GROWTH (SFG)

Measure of an animal's actual physiological growth potential.

Summarizes the information on various physiological rate functions and is a closer approximation of an animal actual growth rate, closely correlating with long-term growth performance

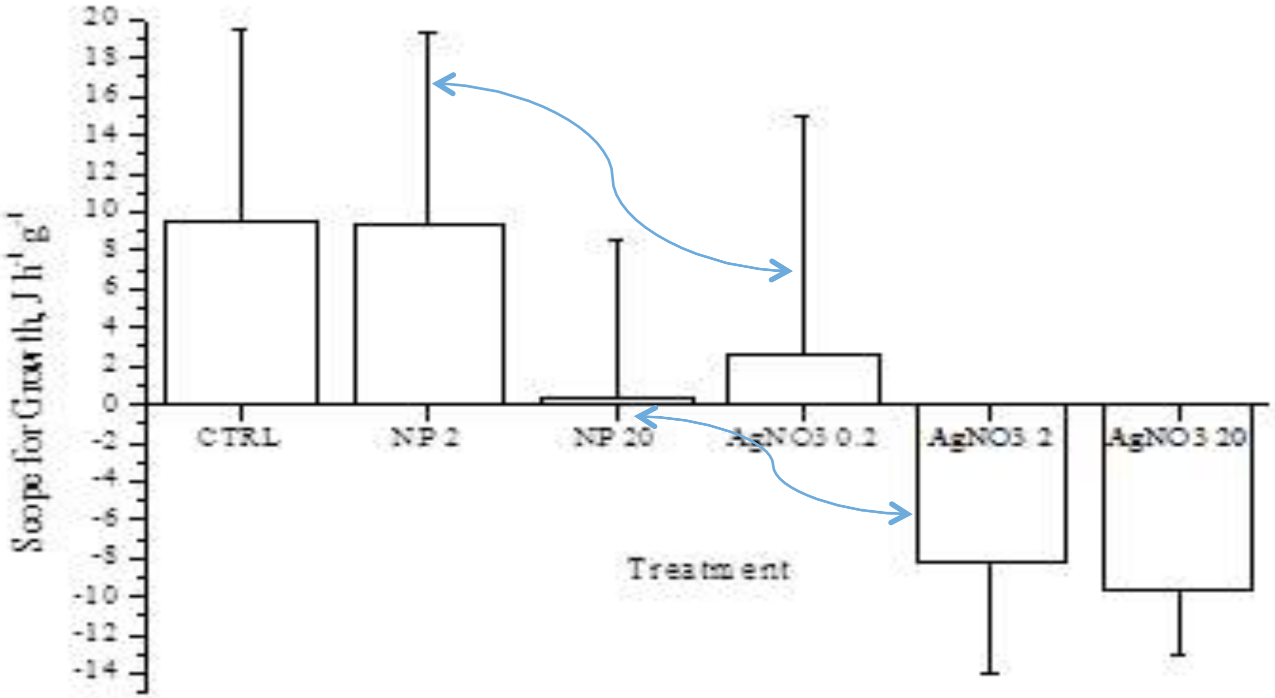
$$C = P + R + E + F$$

C = energy consumed, **P = energy used for animal productivity**, **R** = energy lost in respiratory processes, **E** = energy excreted in dissolved by products, **F** = energy lost in defecation

$$SFG = P = C - (R + E + F)$$

NANOGENTOOLS

Scope for growth

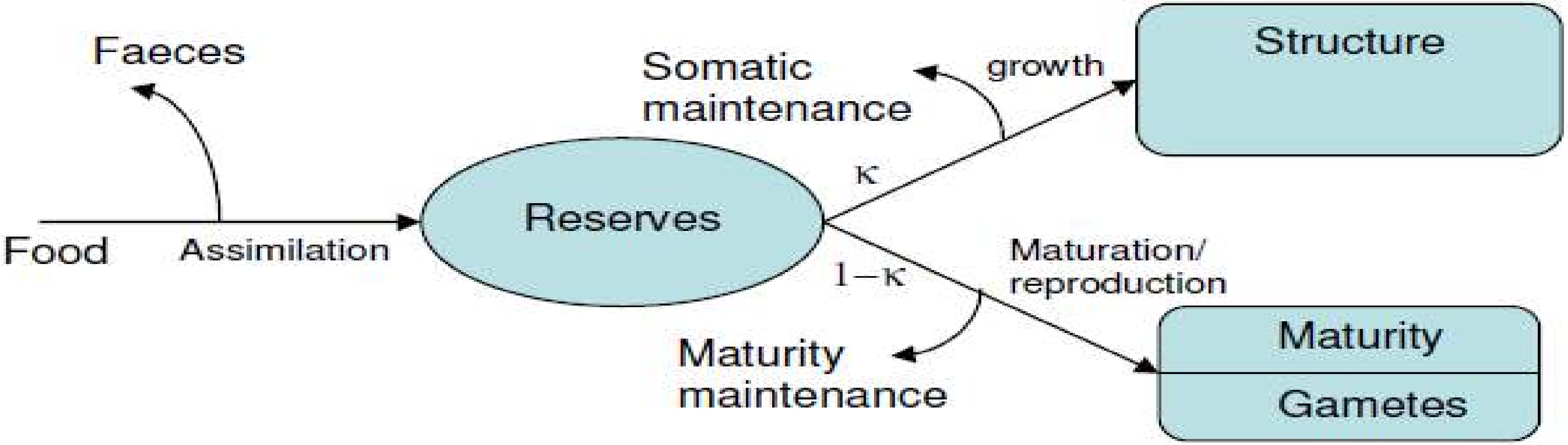


Take home message

- For nanoAg, similar prediction of chronic toxicity test (96h).
- Worse effects for ionic silver.
- Arrows indicate comparable Ag levels according to the prediction model based on the logistic function

Dynamic Energy Budget Theory approach

Prediction of how energy is assimilated and assigned to the different needs for life – growth, development, and reproduction – under fluctuating environmental conditions, assuming ambient food and temperature are known.



Phase I first we need to get DEB parameters of target species and this phase includes both experimental and mathematical and modeling procedures as the covariation method;

Phase II the second step involves a number of experiments in lab mesocosms (hereafter called experimental phase with contaminant) to estimate how functional traits (e.g. feeding, respiration, assimilation rates etc.) change under the contaminant treatment;

Phase III once investigated at which level of the energy budget the target contaminant exerts an effect (e.g. reduction of assimilation or increase the maintenance costs; the third step involves the DEB simulation to predict the potential effect of the target contaminant on two important life history traits as body size and fecundity.

DEB parameters for the effect of Ag ENPs and $AgNO_3$ on the ecophysiological performance of Mytilus galloprovincialis

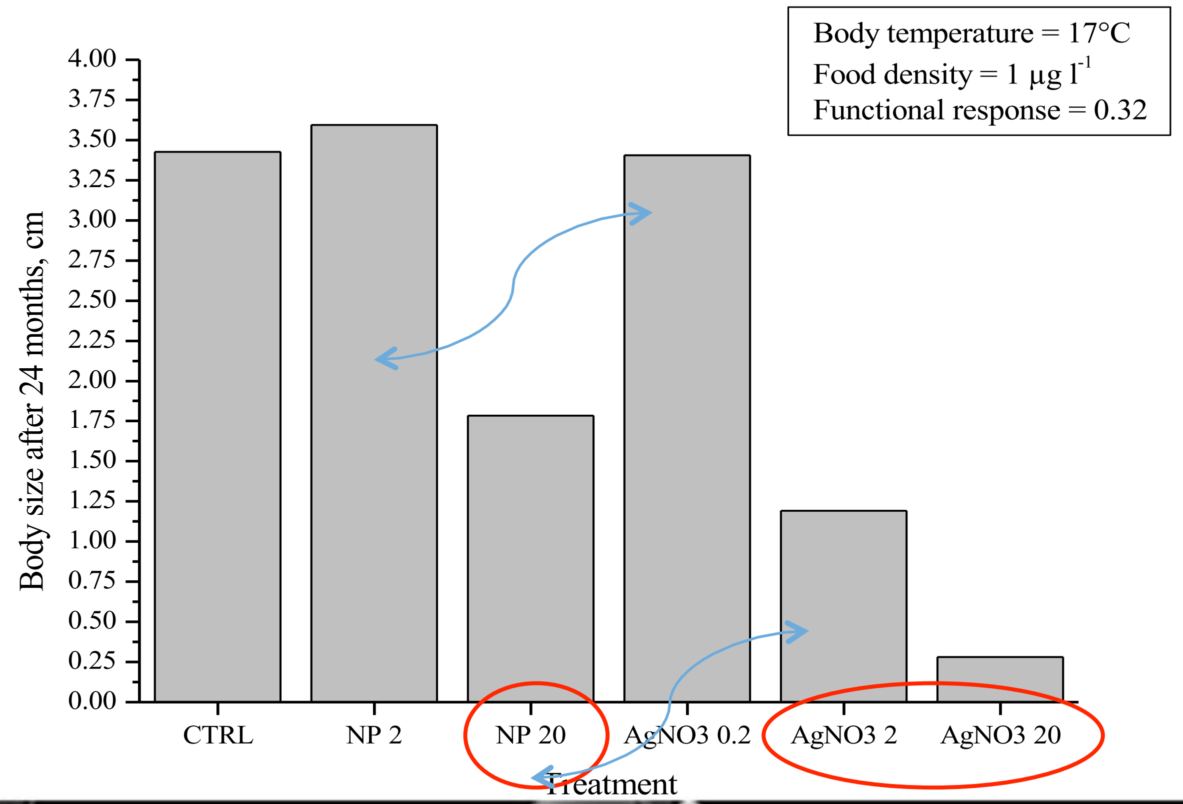
Treatment	AE	% CTRL	Jxm (J h^{-1} cm^{-2})	% CTRL	pM	% CTRL
Control (CTRL)	0.93	-	8.2	-	0.84	-
Ag ENPs 2 µg/L	0.22	-76	43.0	424	0.53	-37
Ag ENPs 20 µg/L	0.14	-85	34.3	318	0.79	-6
AgNO3 0.2 µg/L	0.27	-71	36.1	340	1.36	61
AgNO3 2 µg/L	0.09	-90	36.6	346	1.12	33
AgNO3 20 µg/L	0.06	-94	11.4	39	0.90	8

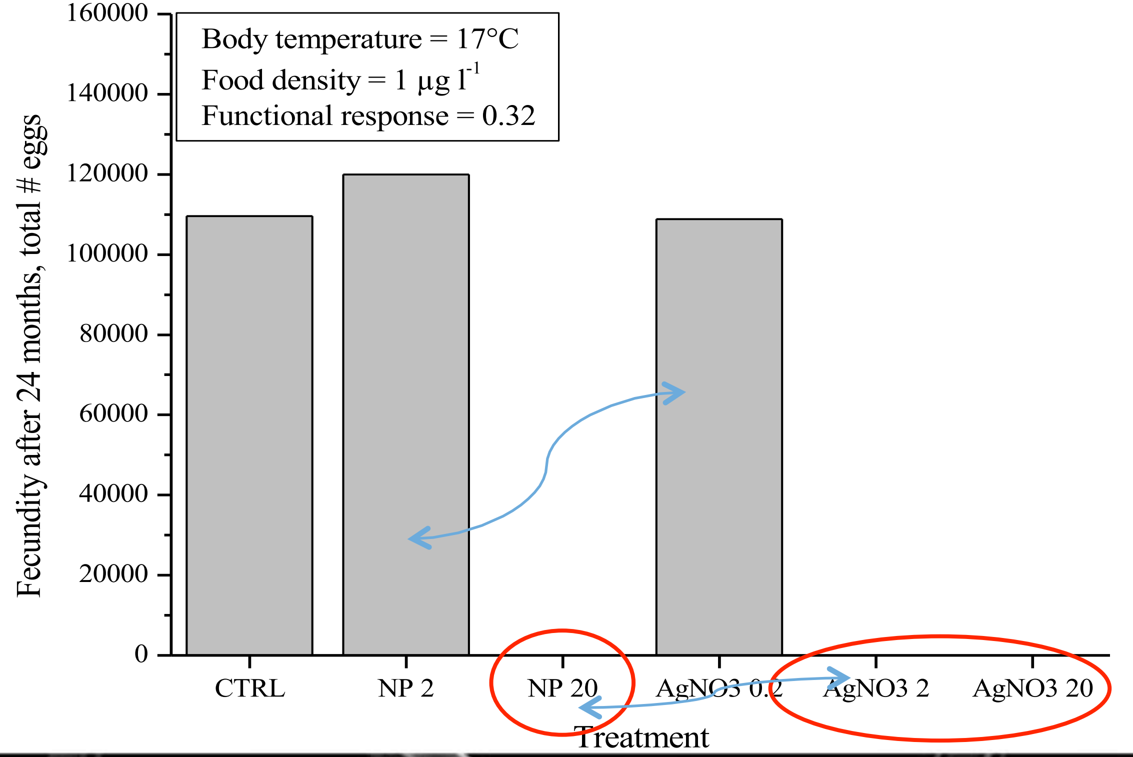
AE absorption efficiency

Jxm maximum ingestion rate for an individual of volume V

pM volume-specific maintenance costs

PREDICTION OF GROWTH AND FECUNDITY





NANOGENTOOLS

Organismic level

Ecotoxicological caracterization of ENPs (acute, subchronic and chronic tests).

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Ecophysiological level

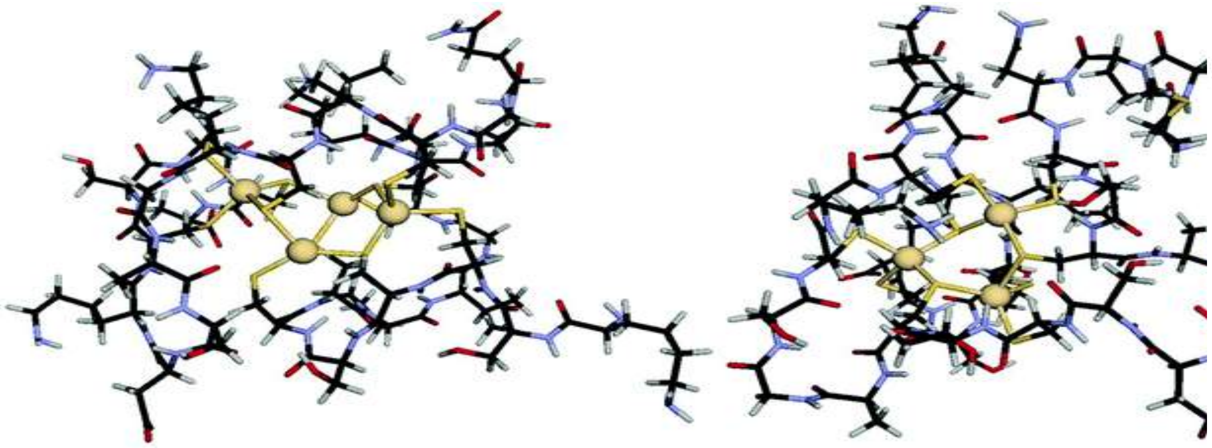
Evaluation of long-term changes in the energy parameters/physiological responses of bivalve.

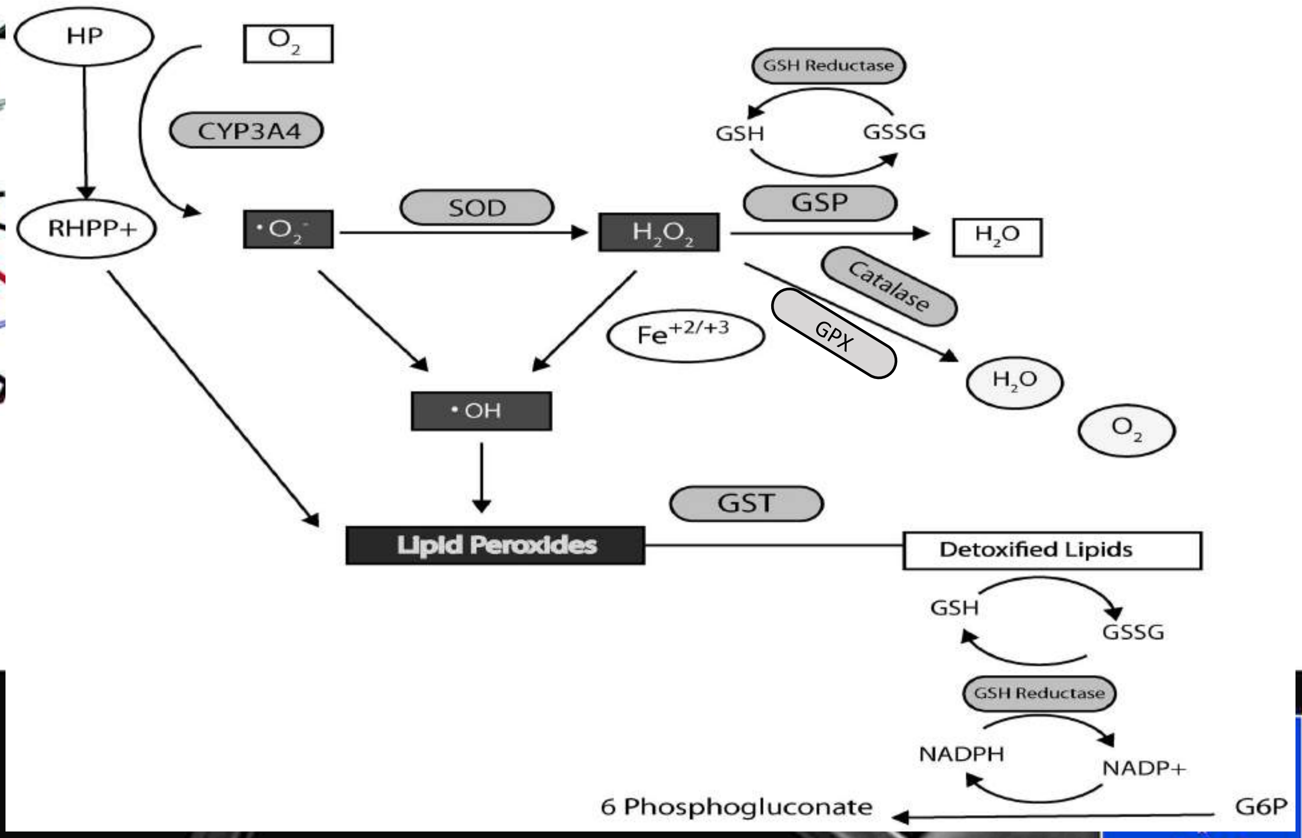
Application of the Dynamic Energy Budget (DEB) model

NANOGENTOOLS

Prior hypothesis:

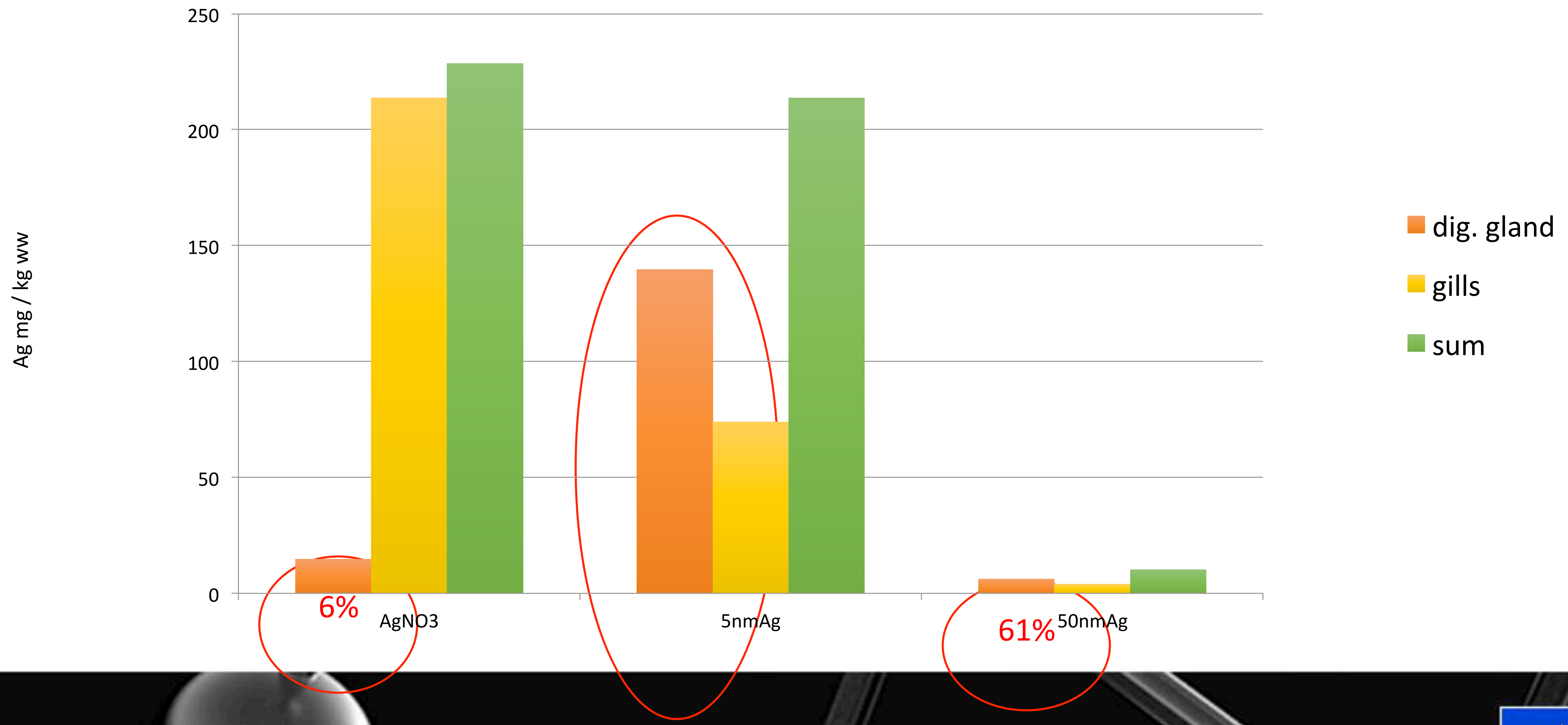
Metals are involved in the generation of free radicals (ROS) that then are responsible for oxidative stress and cellular injuries





SILVER BIOACCUMULATION DYNAMICS

NANOGENTOOLS

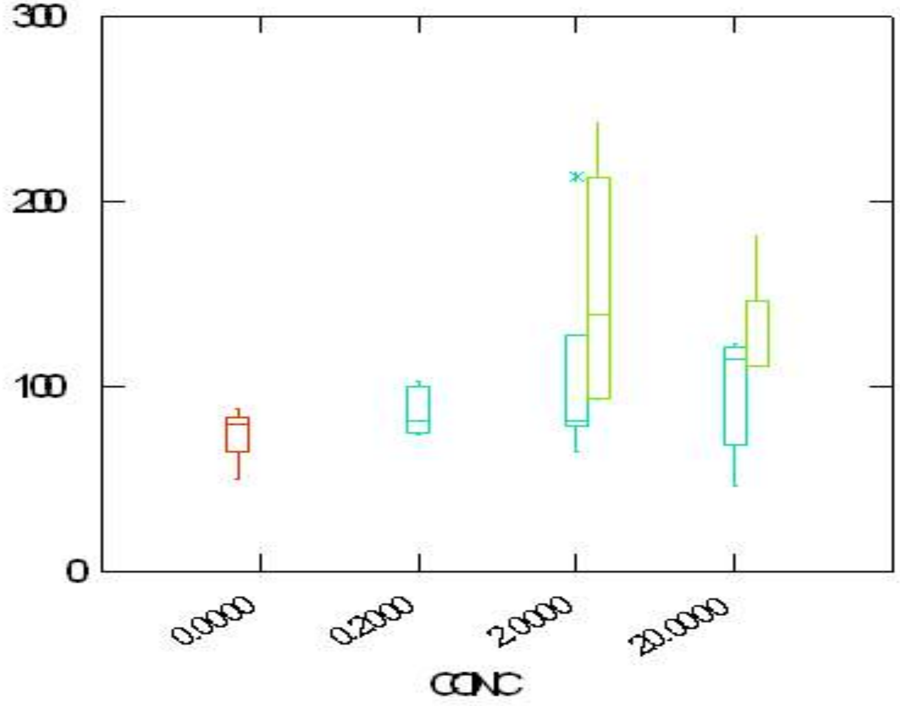


NANOGENTOOLS

METALLOTHIONEIN (protein) CONTENT

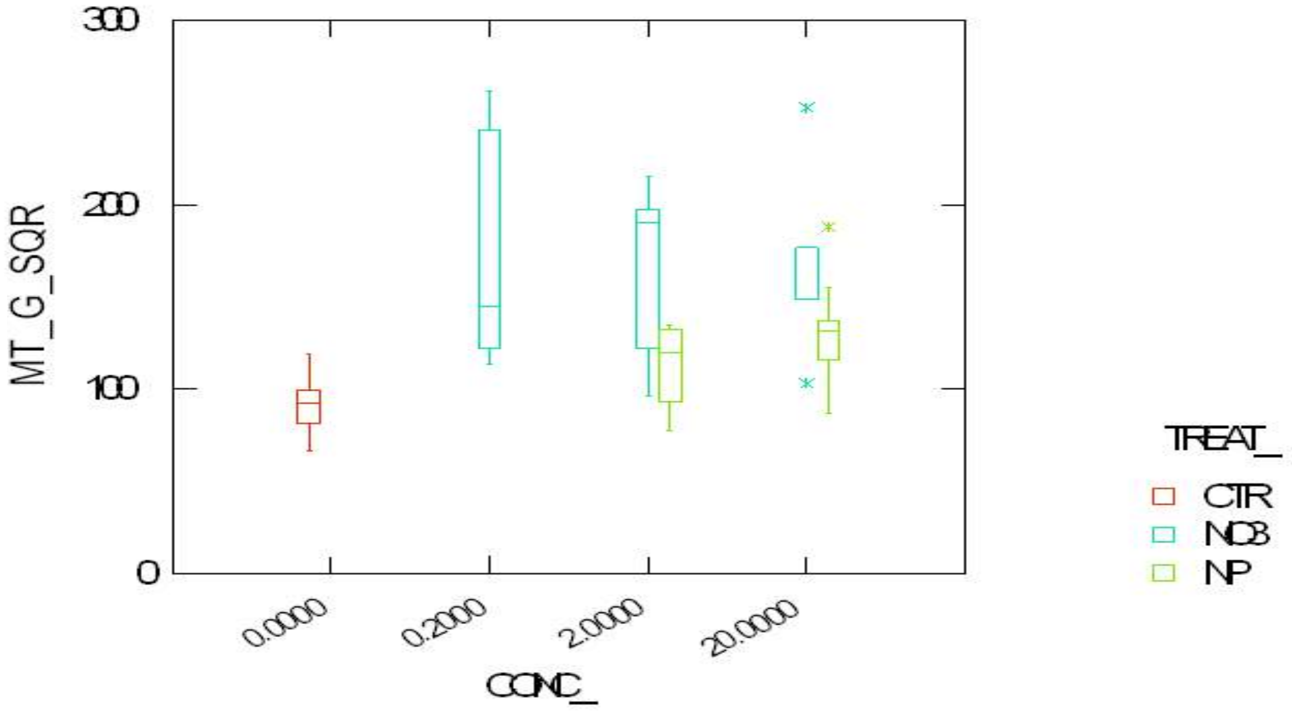
Digestive gland

A.U (sqr)



Gills

A.U (sqr)

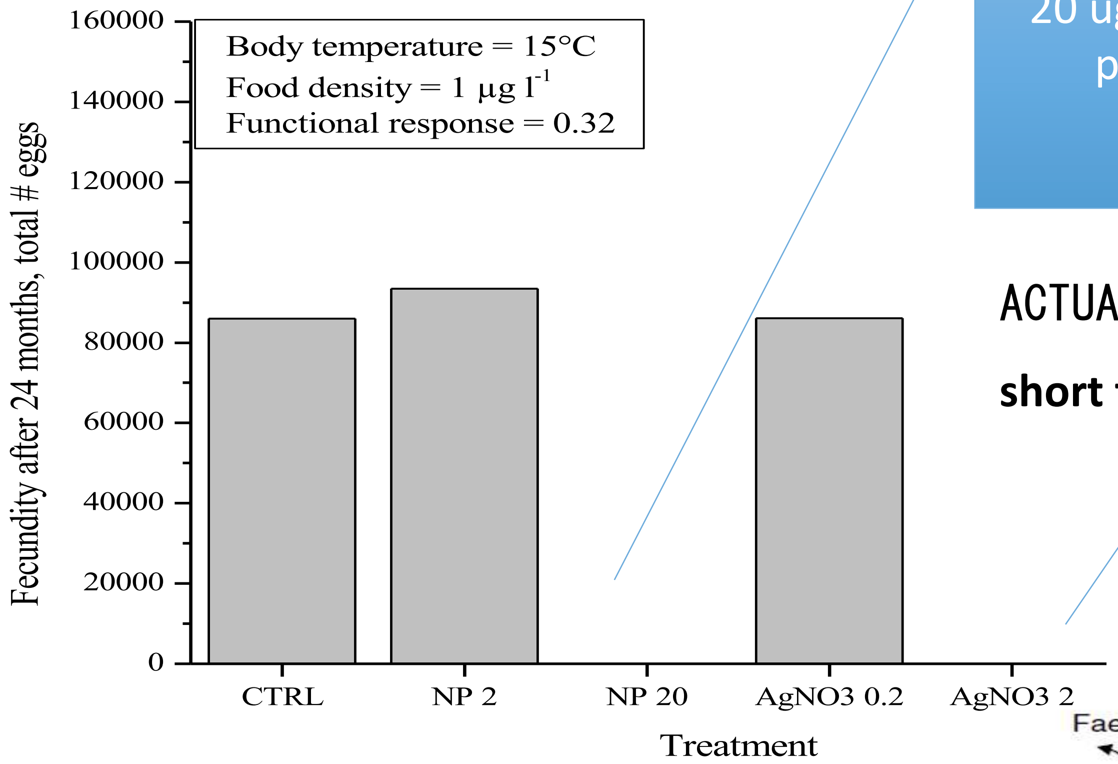


High resolution arrays for Mytilus spp

15 K probes – Agilent sure array technology

- Two –color array for nanoAg and ionic Ag effects in gills
  - T7 cRNA amplification and labelling
  - Common reference (no treatment) design
  - LOESS normalization
  - Linear model for microarray analysis (LIMMA)

Silver effects- nanocosms



20 ug/L nanoAg (5 nm, paraffin coated)
4 weeks

2 ug/L ionic silver
4 weeks

ACTUAL DOSE **1.0 µg/L/h (LOEC)**

short term chronic toxicity test)

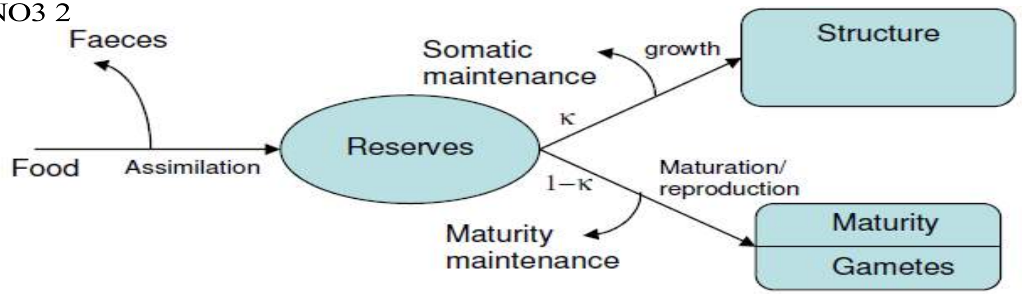
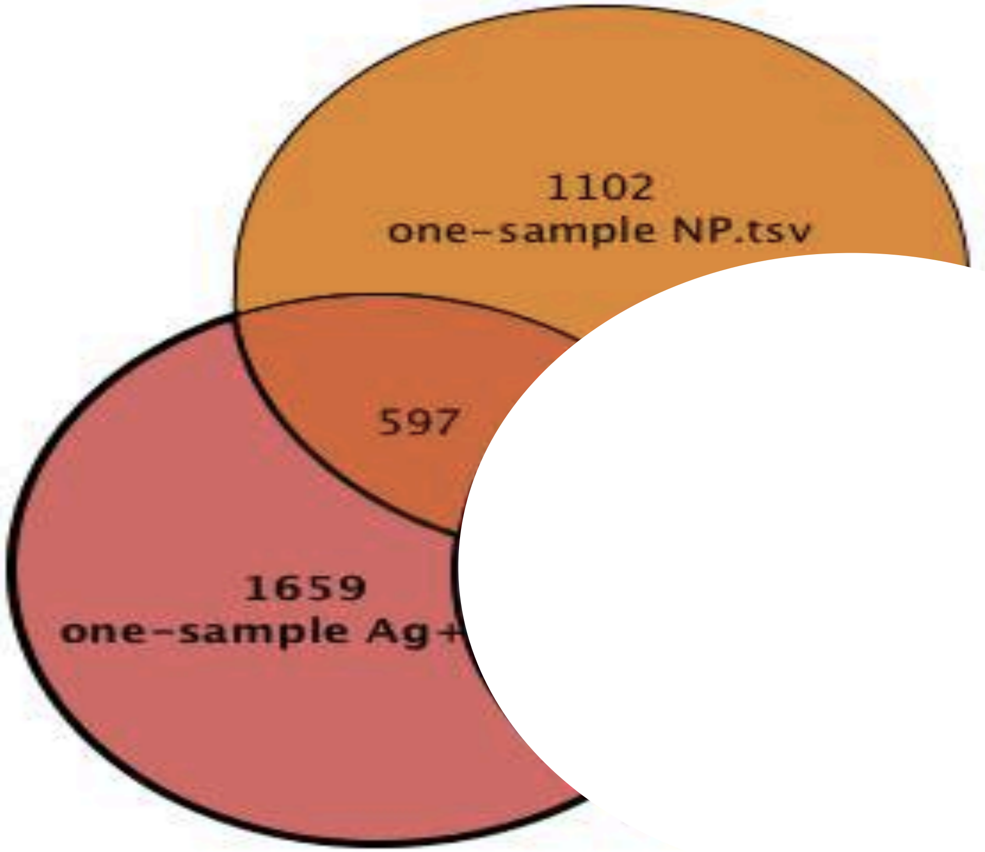


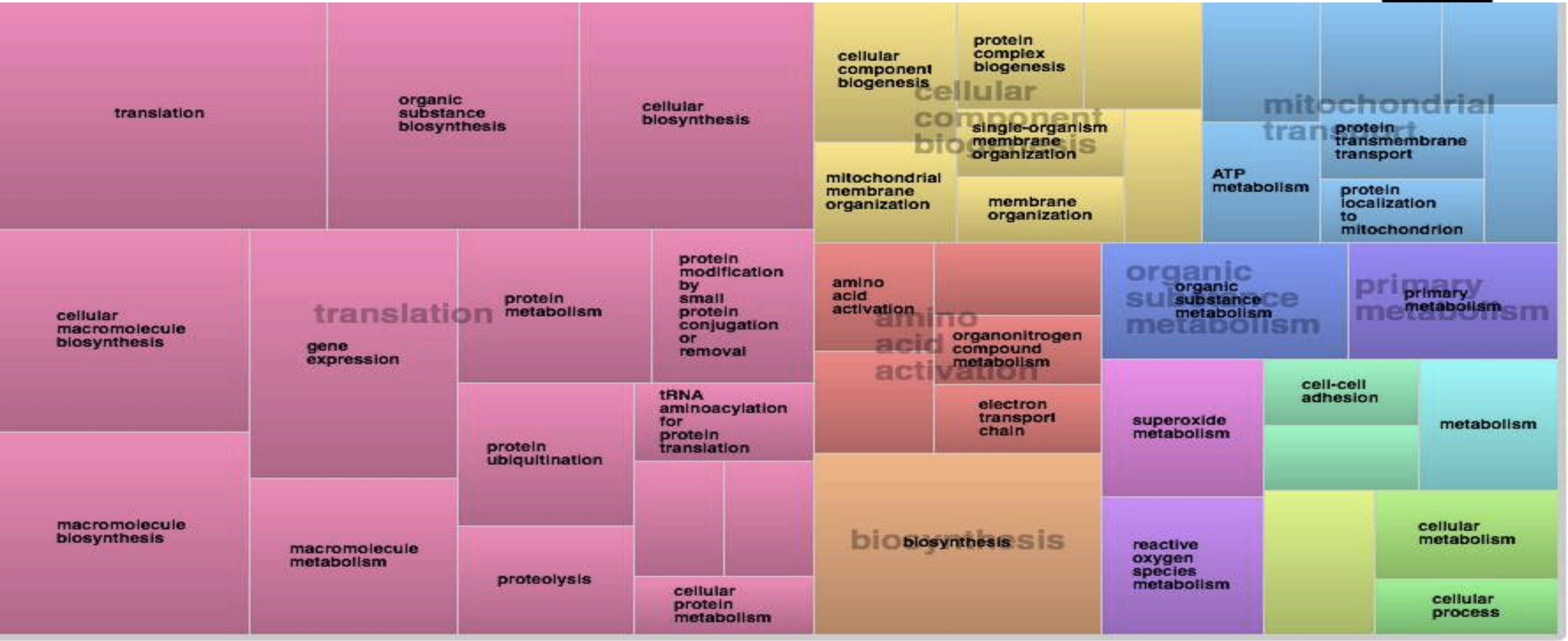
Fig 43 - Representation of the energy fluxes following the DEB approach (Kooijman, 2000)

Gill transcriptomics & high-resolution arrays (GILLS)



Shared genes/features

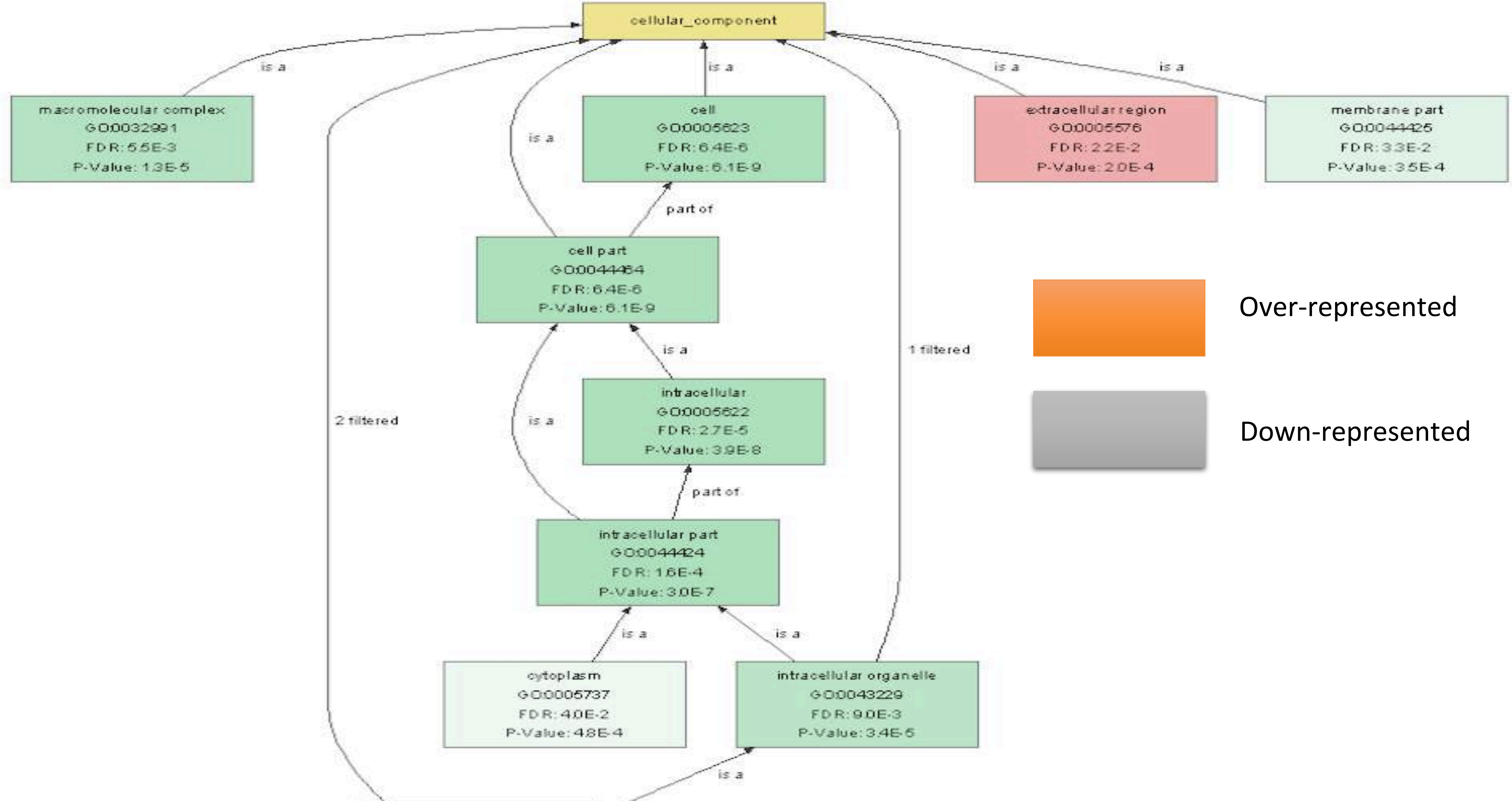
Only in Ag+ Cellular Components



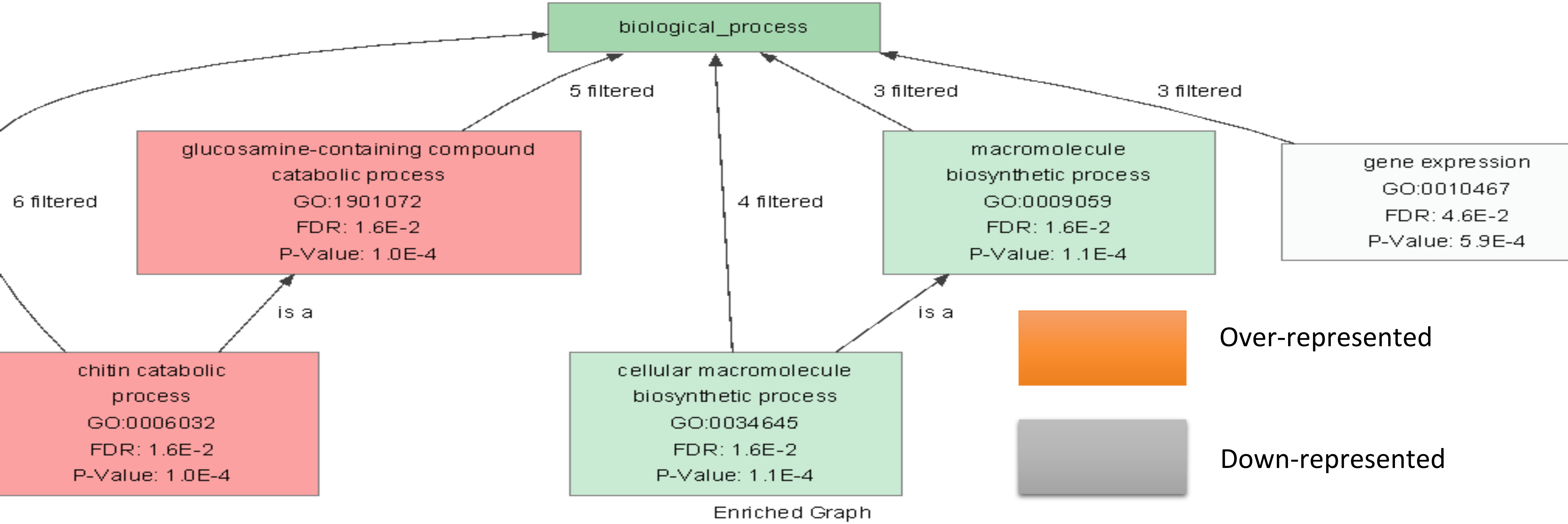
Only in Ag+ CC

Nanogentools confidential

Only in nanoAg (components)



Only in nanoAg (process)



DG NANO


pyridine-containing compound metabolism
seryl-tRNA aminoacylation
membrane lipid metabolism
membrane lipid metabolism
mitochondrial ATP synthesis coupled electron transport
mitochondrial electron transport, ubiquinol to cytochrome c
sphingolipid metabolism
secretion
secretion by cell
secretion by cell
membrane docking

DG ionic silver

NANOGENTOOLS

CONCLUSIONS

1. To what level are silver NPs toxic to mussels ?
2. What is the contribution, if any, of the nano scaled form to toxicity?
3. What is the mode of action of AgENP and what differences with the ionic form?
4. How can AgENP toxicity be projected to population level?

1. To what level are silver NPs toxic to mussels ?

- ✓ The full range of ecotox endpoints has been determined for acute and chronic tests
- ✓ Silver is toxic in the submicromolar range

2. What is the contribution of the nano scale?

- ✓ Our data suggest that actual (total) silver concentration is the leading toxicity driver

- ✓ The main contribution of the nano-scale is relative to the how silver is presented to the animal tissue (aggregation, sedimentation, etc).
- ✓ NanoSilver appears to be less reactive (less ROS activity) than Ag^{+} ions, but more readily available to the digestive tissue

3. What is the mode of action of AgENP and what differences with the ionic form?

- ✓ Ionic silver elicits response to oxidative stress (antioxidant enzymes)
- ✓ Complex dynamics characterize the response to the ionic and nano silver form with a partial overlap of the molecular signature in both analyzed tissues
- ✓ Microarray data indicate Ag^+ had a more ready impact on intracellular targets such as mitochondria and ribosomes (protein translation)

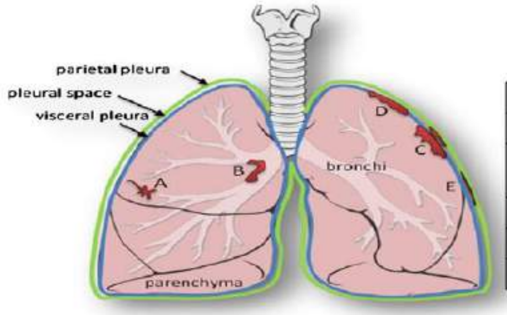
4. Can AgENP toxicity be projected up to high order level?

- ✓ Long term bioenergetic measurements by means of DEB confirmed chronic ecotox endpoints
- ✓ AgNP LOEC for fecundity is around 1 µg/L/(h)
- ✓ PEC/PNEC risk assessment would suggest a risk level of 0.01 (low risk)

Safe by Design and the Fiber Paradigm

MWCNTs

- Nanothinx S.A.
- Nanogentools



Label	Pathology	Site
A	Asbestosis	Parenchyma
B	Bronchogenic carcinoma (lung cancer)	Bronchi
C	Mesothelioma	Pleurae
D	Pleural fibrosis	Pleurae
E	Pleural plaque	Parietal pleura

Fig. 1. Diagram showing the situation of the types of pathology caused by fibres. (*Donaldson et al, Advanced Drug Delivery Reviews 65 (2013) 2078–2086*)

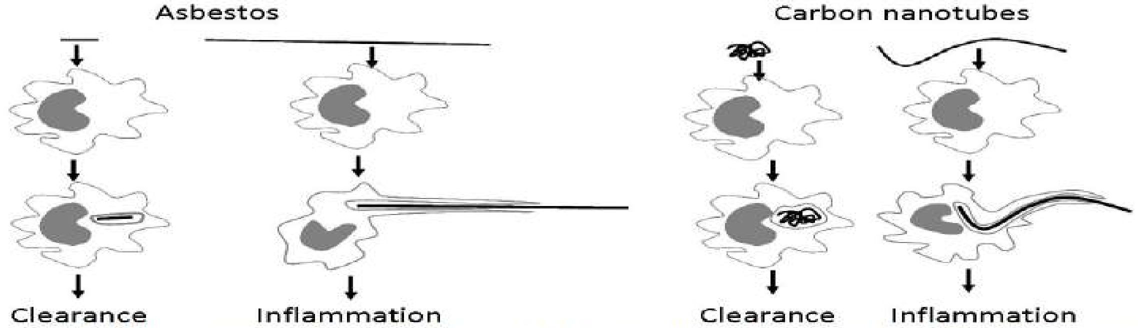
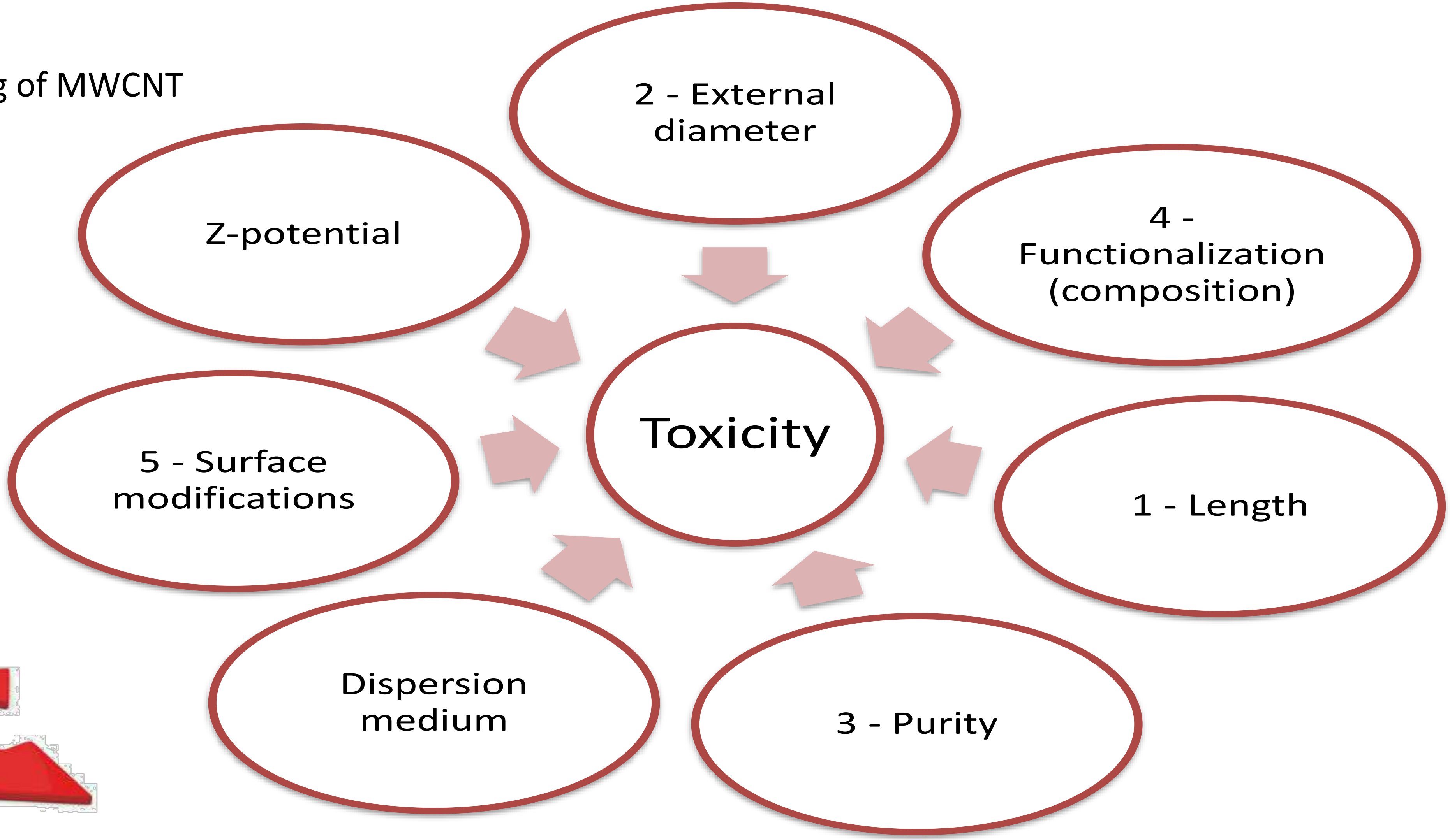


Figure 2 The frustrated phagocytosis paradigm as it relates to long and short fibres of asbestos (left) and various forms of carbon nanotubes (right). When confronted by short asbestos fibres or tangled, compact carbon nanotube 'particles' the macrophage can enclose them and clear them. However the macrophage cannot extend itself sufficiently to enclose long asbestos or long nanotubes, resulting in incomplete or frustrated phagocytosis, which leads to inflammation.

Donaldson et al. Particle and Fibre Toxicology 7 (2010) 5 -22.

Toxicity determinants ?

Mechanistic understading of MWCNT toxicity



Compositional MWCNT library

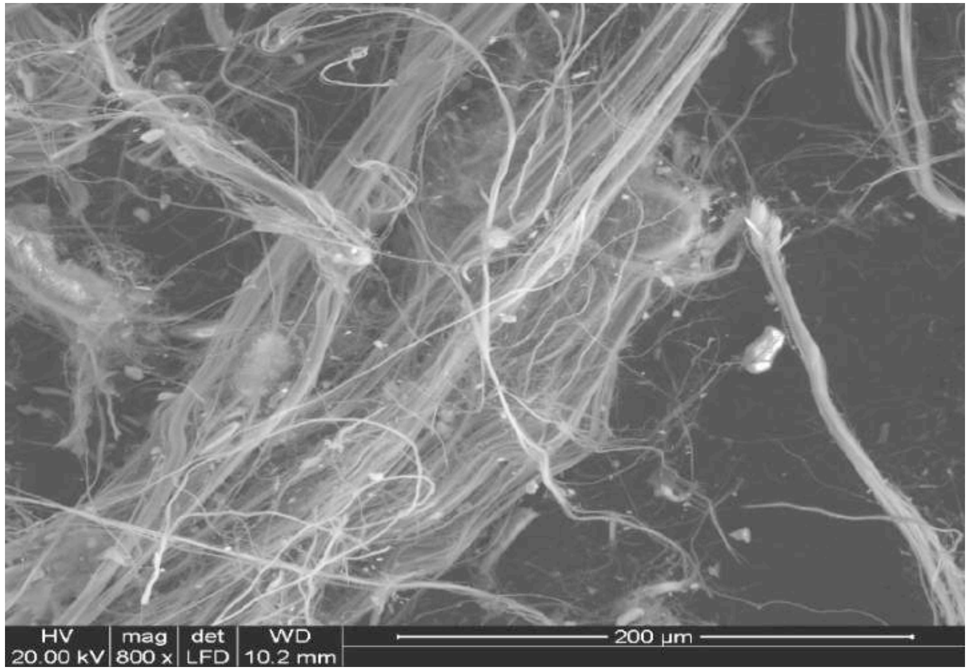
	NTX1 Raw			NTX3 Raw			NTX4 Raw			Number samples
	% Purity	External diameter	Length	% Purity	External diameter	Length	% Purity	External diameter	Length	
Raw material	97	15-35 nm	>10 µm	< 98.5	20-40 nm	>10 µm	<94	6-15 nm	>10 µm	3
Cutting (tip sonication)	97	15-35 nm	3-5 µm 1-3 µm < 1 µm	< 98.5	20-40 nm	3-5 µm 1-3 µm < 1 µm	<94	6-15 nm	3-5 µm 1-3 µm < 1 µm	9
Functionalization -COOH groups (HNO_3 Protocol)	97	15-35 nm	? % funct.	< 98.5	20-40 nm	? % funct.	<94	6-15 nm	? % funct.	3
Purification	-			-			?	6-15 nm	? 1-3 µm Cutting < 1 µm	2

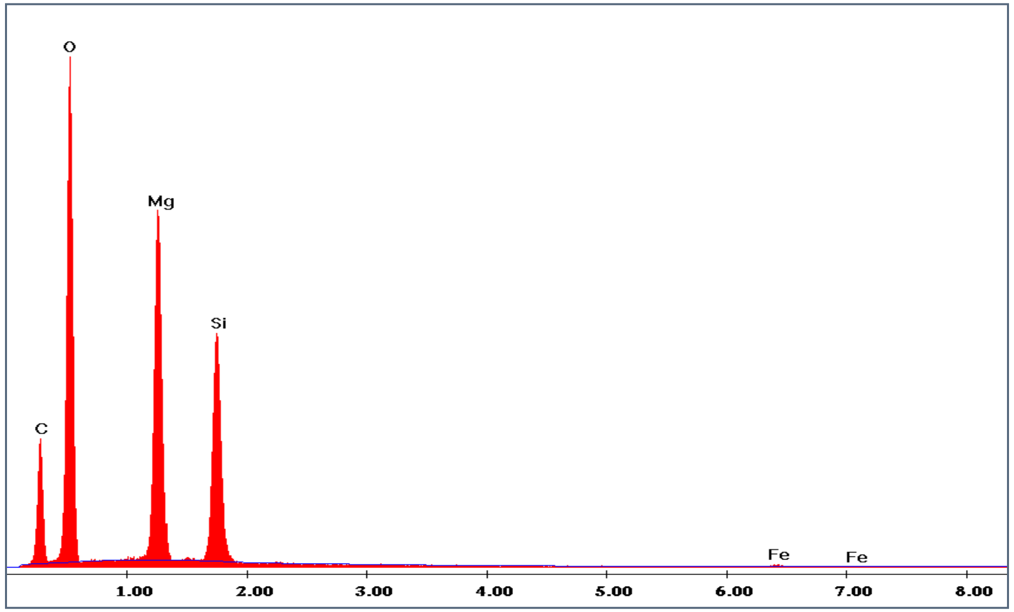
4 sizes, 1 functionalization densities, long and short asbestos
+ NCT-7 (Mitzui) + CB, max 9 conditions for each NTX

? 5 - Surface modifications
Z - potential

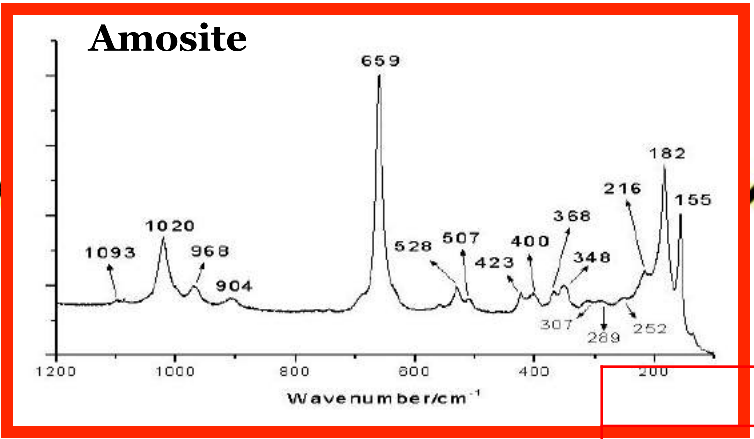
Morphological, Chemical and Crystallographic characterization of asbestos in biological matrix

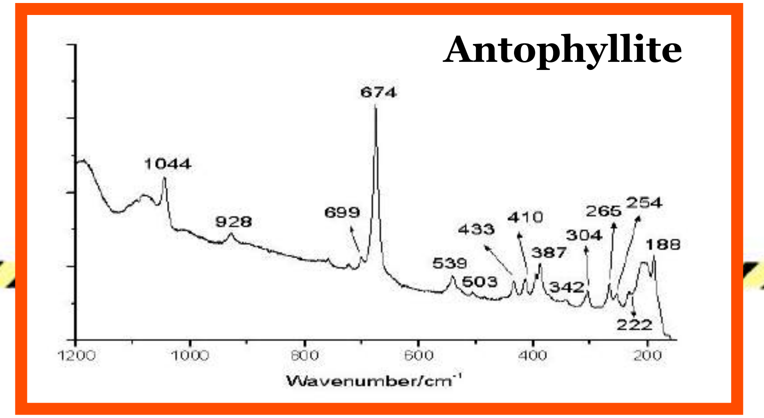
Prof. Caterina Rinaudo, PI @ UPO
Alessandro Croce, Co-PI @ UPO

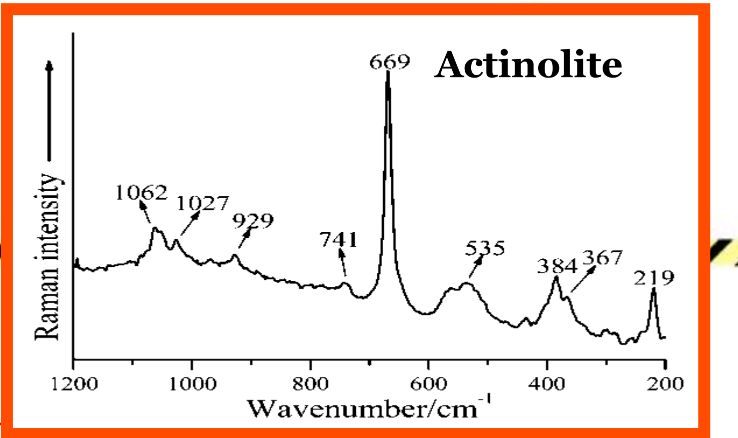




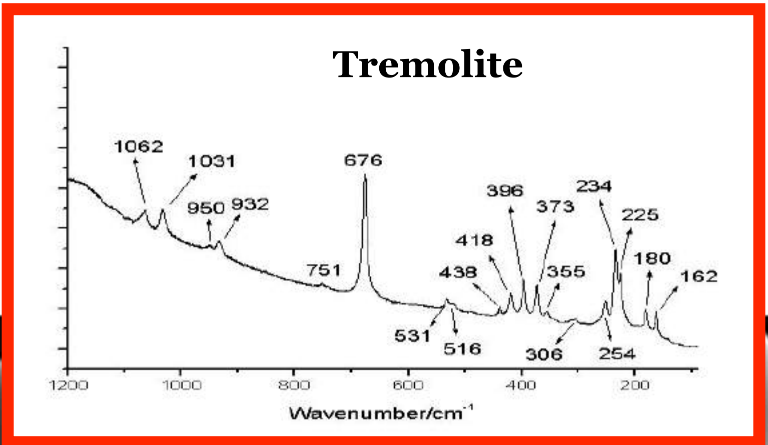
SEM-EDX analysis of asbestos fibers

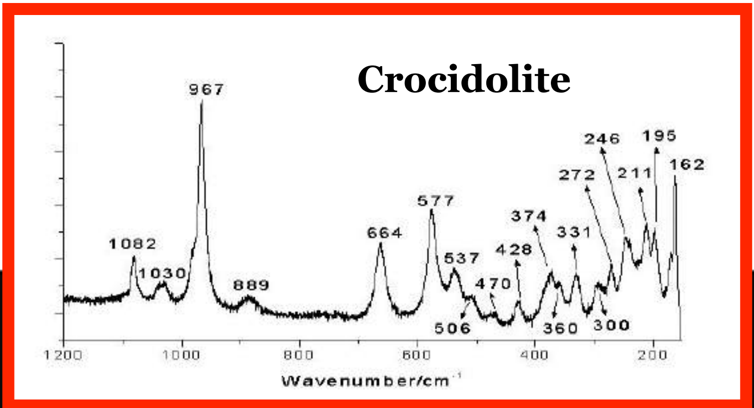


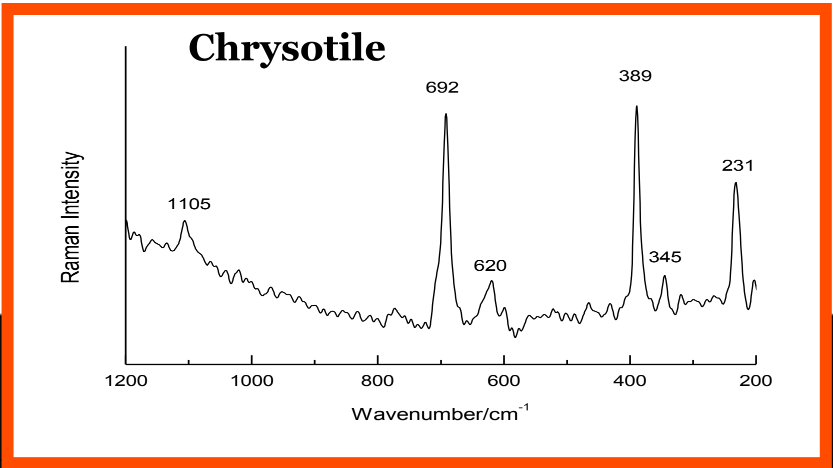




	ν_s	ν_{as}
Amosite	659	1020
Anthophyllite	674	1044
Actinolite	669	1062
Tremolite	676	1062, 1031
Crocidolite	664	1082
Chrysotile	692	1105

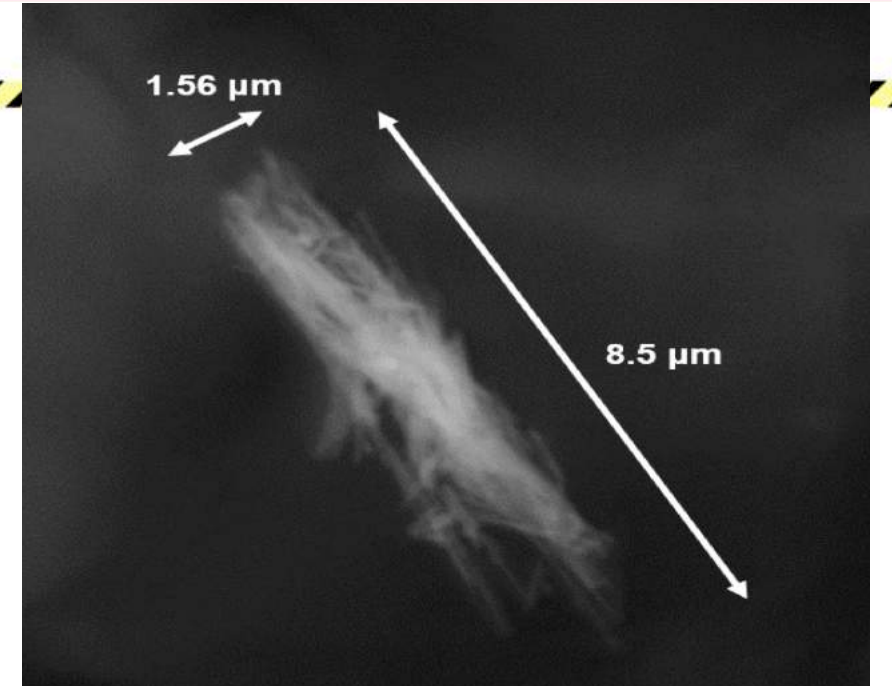


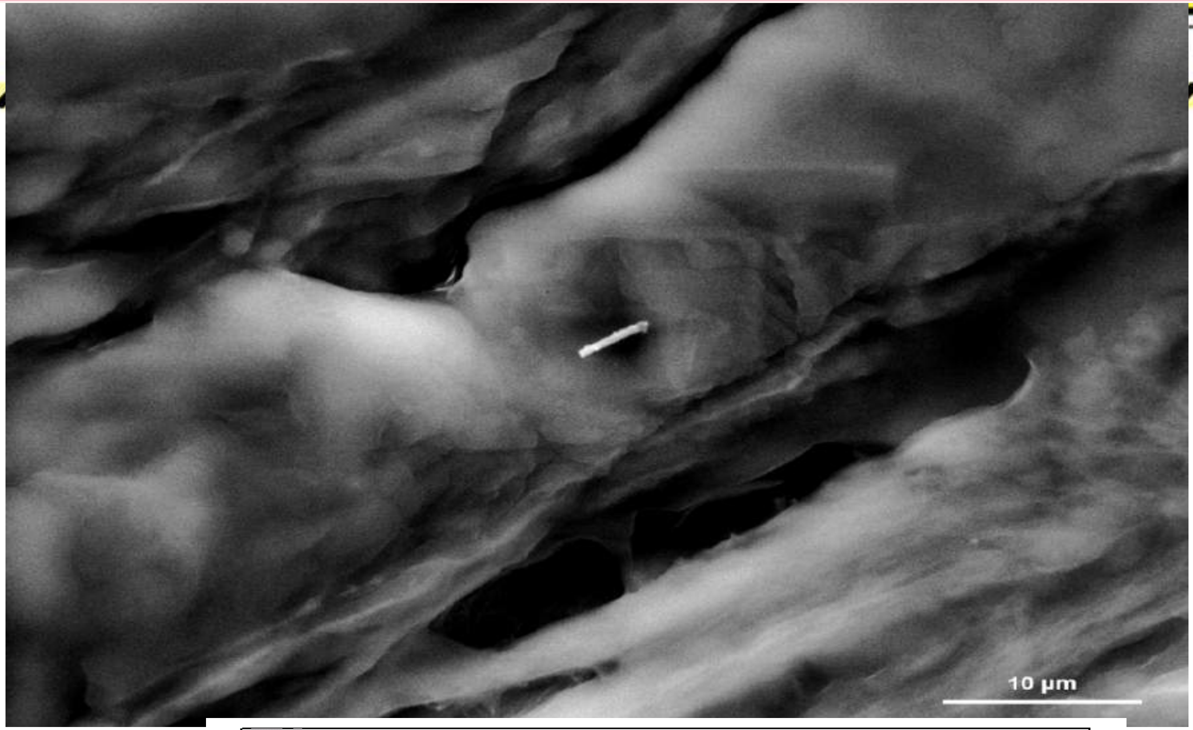


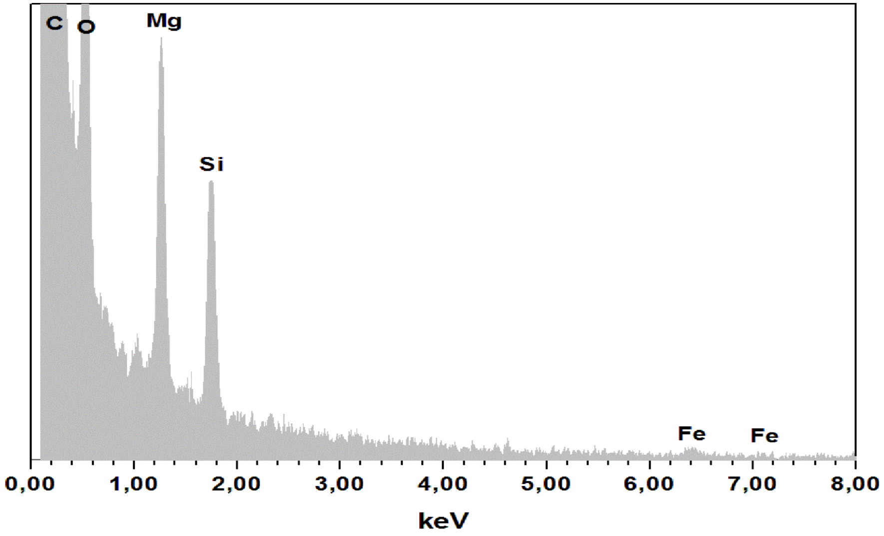


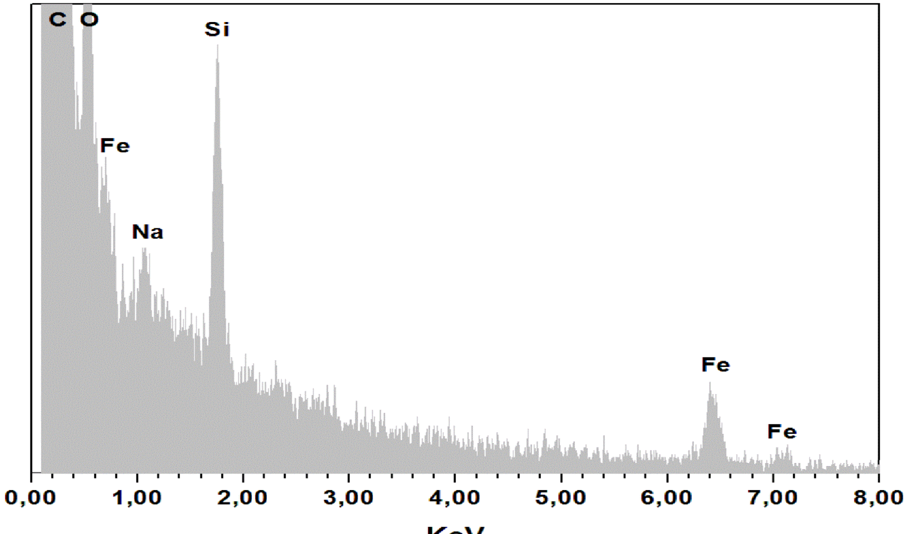
C.Rinaudo, E.Belluso, D.Gastaldi: "Assessment of the use of Raman spectroscopy for the determination of amphibole asbestos" Miner. Mag., 2004, 68(3), 455-465.

TOOLS


1.56 µm
8.5 µm


10 µm


C
O
Mg
Si
Fe
Fe
0,00
1,00
2,00
3,00
4,00
5,00
6,00
7,00
8,00
keV


C
O
Si
Fe
Na
Fe
Fe
0,00
1,00
2,00
3,00
4,00
5,00
6,00
7,00
8,00

Molecular systems approach

- Assess toxicity endpoints, short and long term
  - Survival, mitogenicity, epithelial-fibroblast transition, cell transformation
- Toxicogenomic approach, use classification algorithm
- Detailed Protein corona assessment by Triple-TOF based shotgun proteomics
- Derive a predictive model for asbestos induced lung cancer

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Acknowledgments

HORIZON 2020

SEVENTH FRAMEWORK PROGRAMME

NanOFATE

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Thank you!

The research leading to these results has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 691095.

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